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CHAPTER IV

MANUFACTURE OF TAPIOCA STARCH

1. Importance of Tapioca Starch in the United States. Of the non-cereal starches, tapioca has ranked first in order of importance in the United States. As compared to the 800 million pounds of corn starch and 20 million pounds of potato starch produced in the United States annually, for sale as such, 382 million pounds of tapioca were imported in 1939 and only about 42 million pounds of all other starches, such as arrowroot, sago, rice, and potato (1). Owing to the present disruption in world trade, the market position of tapioca, which was apparently maintained in 1940 and the first part of 1941, has recently

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CHAPTER XIX

USES OF STARCH AND STARCH PRODUCTS IN THE FERMENTATION INDUSTRIES

LEO M. CHRISTENSEN

1. Introduction. The fermentation industries constitute a branch of chemical manufacture in which starches and sugars are the principal raw materials. These are converted by the use of the enzymes in yeasts, molds, and bacteria into a number of useful chemicals. The best known and the one of greatest commercial interest is ethyl alcohol, but other fermentation chemicals, such as *n*-butanol, acetone, lactic acid, citric acid, gluconic acid, glycerol, and 2,3-butylene glycol, are also of large present or potential commercial importance. Many other chemicals of commercial interest can be made by the action of micro-organisms upon carbohydrates, and there is a growing industrial interest in such processes. Fulmer and Werkman (1) have presented an excellent index to these fermentative reactions.

As in other branches of chemical manufacture, the fermentation industries have used the raw material at hand that costs least. During the past 25 yrs., the carbohydrate of lowest cost suited for use in fermentation processes has been blackstrap molasses, a by-product from the manufacture of cane sugar, for which there has been no other large market. Because it is a by-product, it has been sold at a price it brings in the competitive market, and it has been available at a level just below the average price of corn and other competitive raw materials. The fermentation industries have been using about 2 million tons of blackstrap molasses each year, which amount is nearly all of the total world production.

Recently (1942), the raw material situation changed considerably. First, it has been found difficult to transport supplies of foreign raw materials during periods of a disruption in world trade. Second, the demand for the fermentation chemicals has expanded to the extent that there is not enough blackstrap molasses produced to supply the needs. During 1942, the use of grains in the fermentation industries expanded materially and a continued expansion seems assured for the

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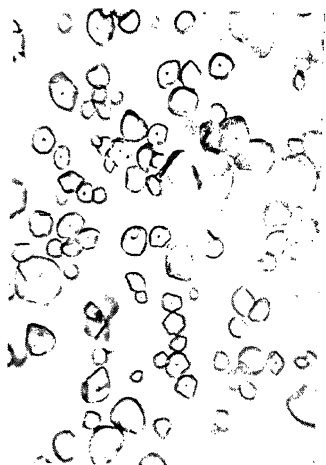


FIG. 5. Sweet potato starch.

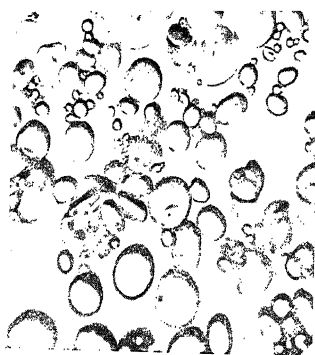


FIG. 6. Wheat starch.



FIG. 7. Barley starch.

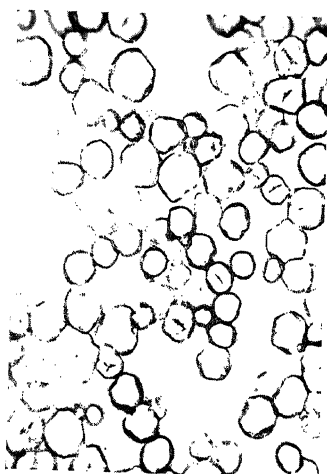


FIG. 8. Waxy sorghum starch.

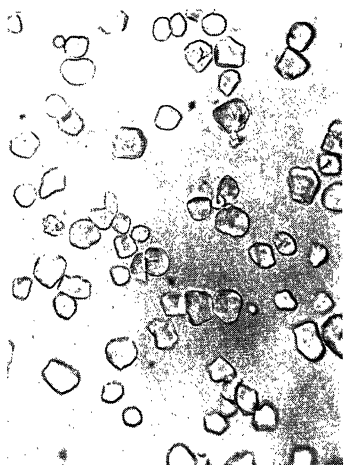


FIG. 9. Waxy maize starch.

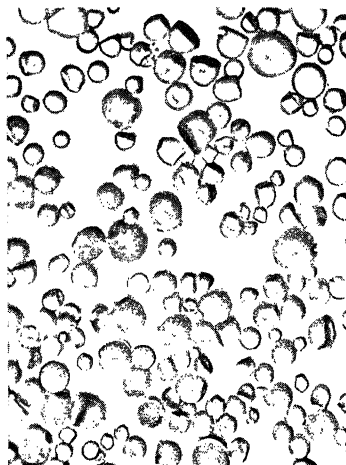


FIG. 10. Tahiti arrowroot starch.

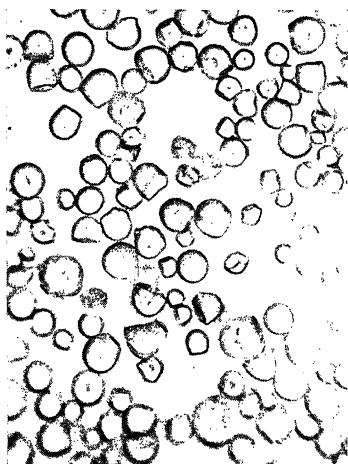


FIG. 11. Tapioca starch.

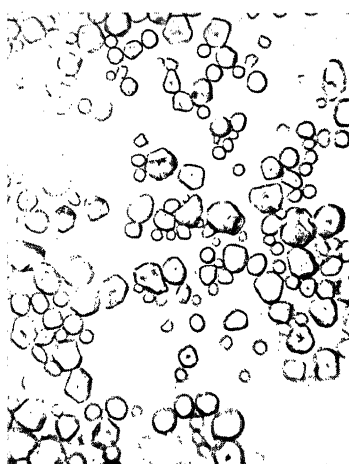


FIG. 12. Corn starch.

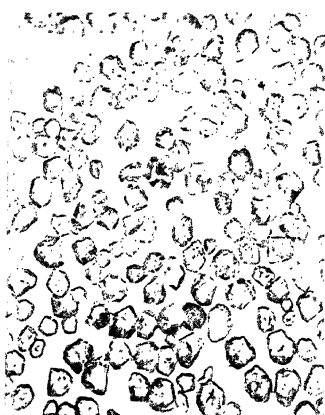


FIG. 13. Corn starch (horny); Hybrid 998, Kutias and Illinois utility.

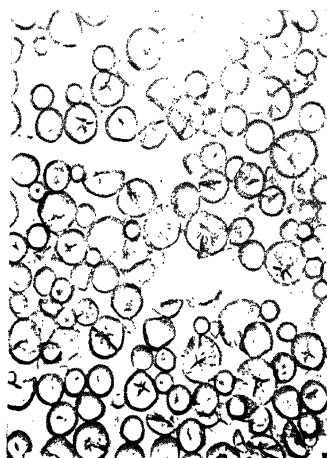


FIG. 14. Corn starch (floury); Mandan.

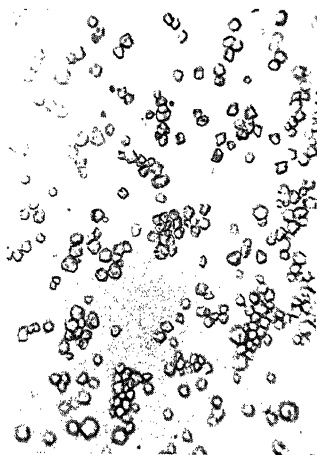


FIG. 15. Rice starch.

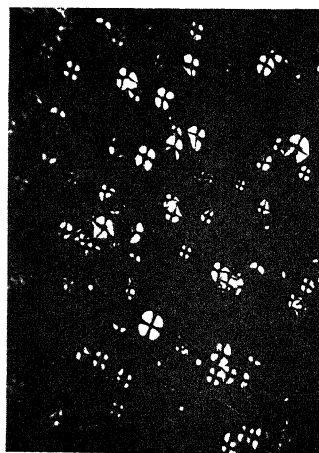


FIG. 16. Tapioca starch in polarized light.

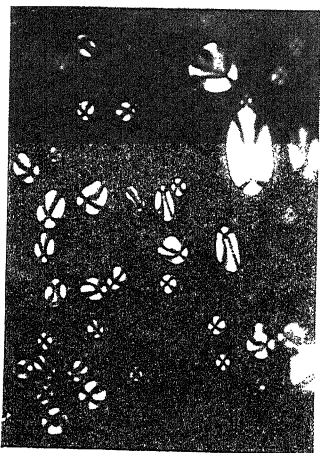


FIG. 17. Potato starch in polarized light ($\times 100$).

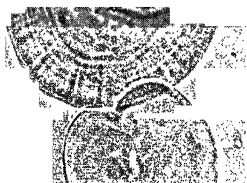


FIG. 18. Rye starch in water at 55° C. ($\times 450$).



FIG. 19. Potato starch in water at 65–70° C. ($\times 100$).



FIG. 20. Acid-modified potato starch in water at 65–70° C.



FIG. 21. Acid-modified potato starch in water at 65-70° C.

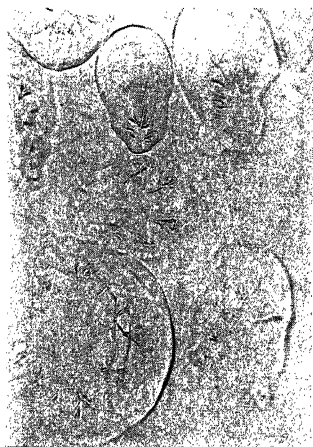


FIG. 22. Canna starch in water at 65-70° C.



FIG. 23. Sweet potato starch in water at 65-70° C.



FIG. 24. Tapioca starch in water at 65-70° C.

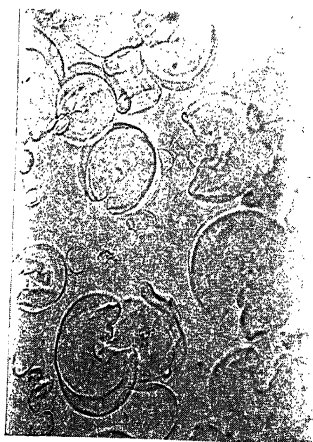


FIG. 25. Rye starch in water at 65-70° C.



FIG. 26. Sago starch in water at 65-70° C.



FIG. 27. Corn starch in water at 80-90° C.

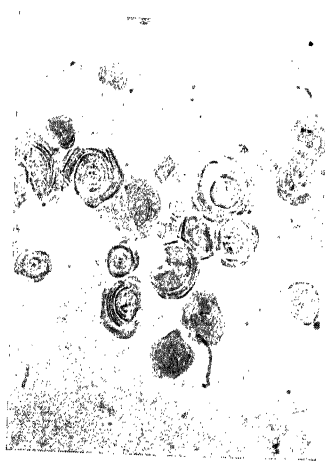


FIG. 28. Torrefaction dextrin from corn starch; early stage of solution in water-glycerol mixture.

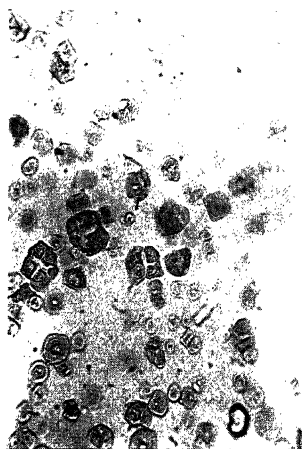


FIG. 29. Gelatinized, thin boiling corn starch.

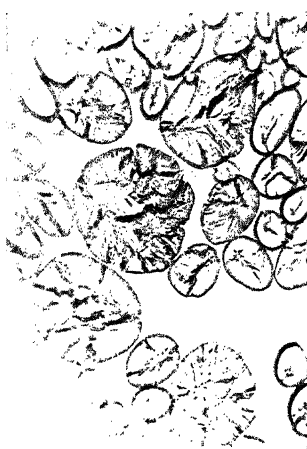


FIG. 30. Potato starch granule, crushed by pressure.

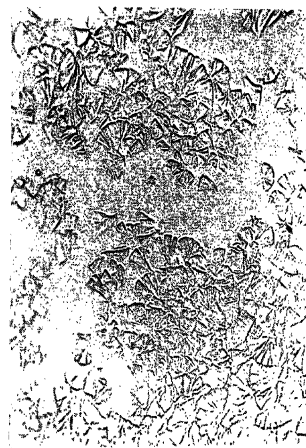


FIG. 31. Thin boiling potato starch, crushed by pressure.

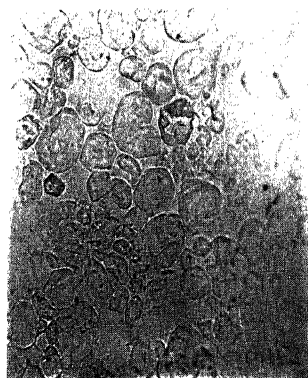


FIG. 32. Corn starch, dry ball-milled and suspended in cold water.

magnitude, and are definitely more stable in water solution even after repeated recrystallizations with butanol to effect their complete purification.

To explain these results, it might be assumed that the orientation of linear chains into products which tend to become insoluble is facilitated as the chain

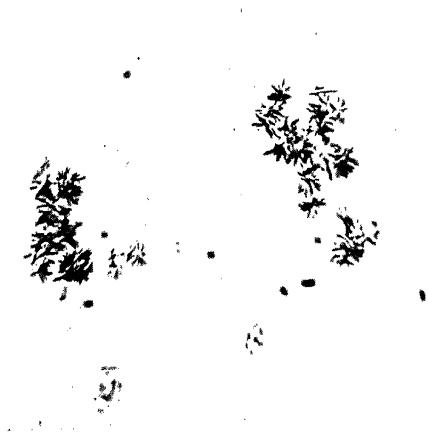


FIG. 55. Potato, crystalline amylose ($\times 300$)

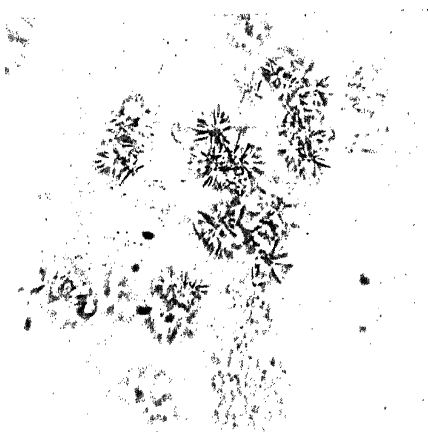


FIG. 56. Tapioca, crystalline amylose ($\times 300$)

of maltose residues becomes longer, but that, after a certain chain length is reached, intermolecular cross-bonding, at a sufficient number of points to reduce the water solubility of the product becomes more difficult, owing to the unwieldy and serpentine behavior of very long chains in solution. This theory does not

TABLE XIX
Retention of Plasticizer by Starch Acetate Films

Plasticizer	Viscosity of ester	Original content of plasticizer	Loss in heating	Loss on leaching
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. None	Low	00.00	5.28	14.96
2. "	High	00.00	6.89	13.45
3. Dimethyl phthalate	Low	10.60	9.80	11.95
4. " "	High	8.64	7.72	14.09
5. Diethyl phthalate	Low	9.46	5.16	11.62
6. " "	High	8.85	7.82	13.12
7. Di- <i>n</i> -propyl phthalate	Low	9.67	5.53	10.04
8. " "	High	7.82	5.24	10.16
9. Diisopropyl "	Low	9.62	4.43	12.63
10. " "	High	8.52	4.24	9.66
11. Dibutyl phthalate	Low	7.64	4.30	11.20
12. " "	High	12.98	9.97	10.82
13. Diamyl "	Low	11.62	5.46	10.28
14. " "	High	12.11	5.94	8.21
15. Diphenyl phthalate	Low	9.48	5.59	11.68
16. " "	High	8.89	6.67	9.78
17. Dibenzyl "	Low	10.79	6.57	9.65
18. " "	High	8.82	5.51	10.28
19. Dimethoxyethyl phthalate	Low	11.32	7.20	12.52
20. " "	High	9.78	6.84	12.02
21. Diethoxyethyl phthalate	Low	9.85	7.21	10.71
22. " "	High	11.90	8.02	10.26
23. Dibutoxyethyl phthalate	Low	11.53	3.59	9.35
24. " "	High	12.18	4.47	8.88
25. Triethyl phosphate	Low	9.39	6.20	14.08
26. " "	High	9.35	8.00	15.19
27. Triphenyl phosphate	Low	8.74	4.21	10.52
28. " "	High	10.74	5.32	11.53
29. Tributyl phosphate	Low	17.79	3.50	5.44
30. " "	High	11.38	8.11	9.12
31. Tricresyl phosphate	Low	10.95	4.18	11.90
32. " "	High	10.22	3.62	8.52
33. Tributyl citrate	Low	8.61	4.03	9.21
34. " "	High	11.99	5.23	8.23
35. Acetyl triethyl citrate	Low	9.84	4.62	9.65
36. " " "	High	8.70	4.04	9.37
37. Dibutyl tartrate	Low	9.44	3.88	11.20
38. " "	High	8.62	7.27	15.40
39. Diethyl "	Low	10.63	3.62	17.81
40. " "	High	10.93	7.14	11.35
41. Methyl- <i>o</i> -benzyl benzoate	Low	9.41	6.85	15.58
42. " " "	High	10.55	6.38	14.48
43. Ethyl- <i>o</i> -benzyl benzoate	Low	9.03	4.24	14.17
44. " "	High	9.59	12.02	13.88
45. Triphenyl guanidine	Low	10.18	3.09	12.24
46. " "	High	10.79	6.22	11.98
47. Triacetin	Low	12.01	7.03	17.50
48. "	High	11.72	6.29	21.40

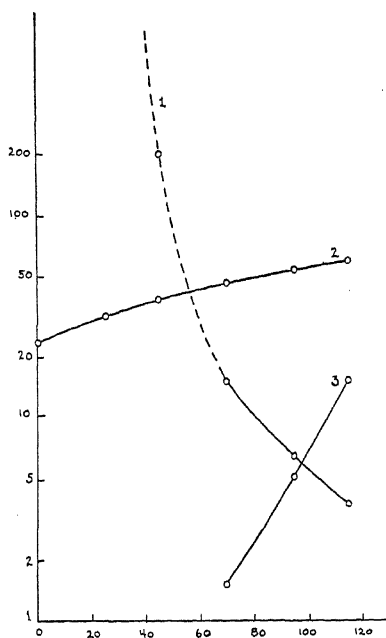


FIG. 77. Corn, white dextrin conversion. Curve 1, viscosity (centistokes), Curve 2, alkali lability, and Curve 3, solubility (per cent) plotted *versus* the time of conversion in minutes

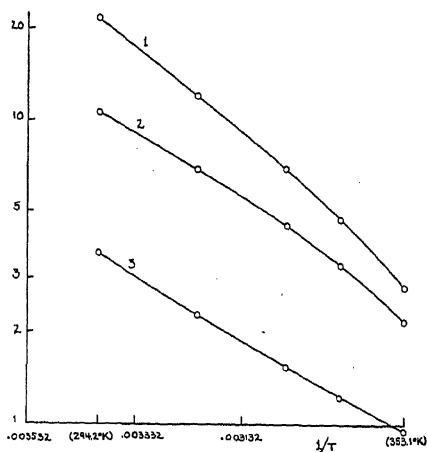


FIG. 78. Viscosity (centistokes) *versus* $1/T$ for Curve 1, white dextrin, 3% soluble; Curve 2, white dextrin, 4% soluble; Curve 3, white dextrin, 20% soluble.

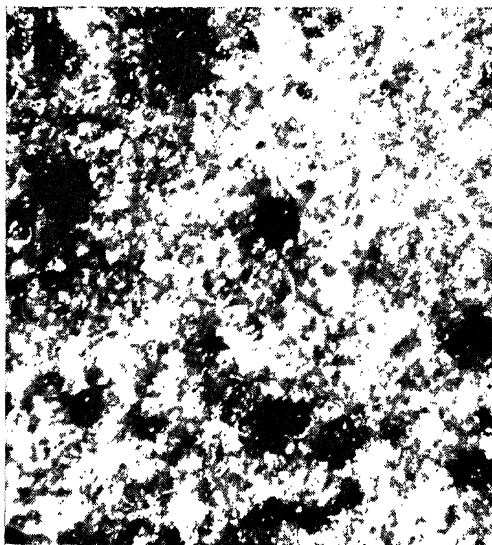


FIG. 94. Iodine-stained sheet made by adding 2% of a roll-dried corn starch product to the pulp at the beaters; 80% retention; 7% increase in the Mullen test for bursting strength; photographed at a magnification of 100.

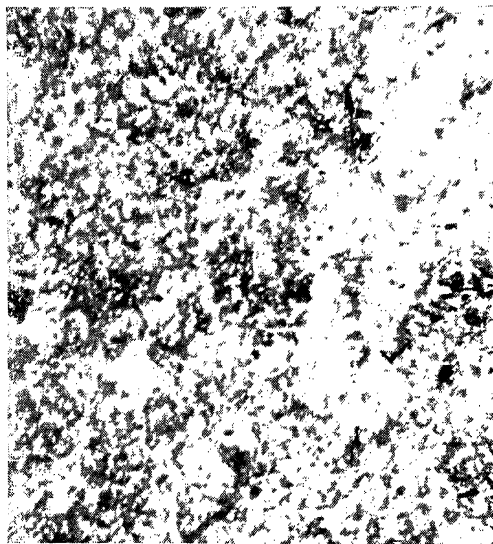


FIG. 95. Iodine-stained sheet made by adding 2% of a roll-dried, chlorine-treated corn starch product to the pulp at the beaters; 64% retention; 20% increase in the Mullen test for bursting strength; photographed at a magnification of 100.

PREFACE

The purpose of this text is to review and bring up to date our accumulated knowledge of starch and of the more important products derived therefrom. Considerable progress has been made in recent years in many phases of the subject, both fundamental and applied. The picture of the ultimate organization of the starch granule, of the chemical structure of starch molecules, and of its behavior in solution or dispersion, in particular, has been materially clarified by recent research. Undoubtedly, our knowledge of the starches is far from complete. The application of starch products in industry, particularly chemical technology, is still in its infancy. The beginner in carbohydrate chemistry should be warned, therefore, not to misinterpret our effort towards coordination by forming the opinion that further investigations are not required or that the subject has reached a final stage. Rather, it is hoped that he will see the possibilities for further fundamental research with a clearer vision and the possibilities for new industrial applications of this almost inexhaustible supply of raw material.

It is also hoped that this survey will be of benefit not only to the carbohydrate chemist but to the technologist, whose daily task is concerned with more efficient production of starch products and with their more efficient utilization. Because these are the primary objects, the manner of presentation of the subject is that of descriptive chemistry. Possibly a kinship between the approach to the study of these substances encountered in elementary chemistry and the approach to the study of starch will be more readily apparent.

The text is not necessarily intended to be comprehensive. Full justice could not be done in one volume to many important divisions of the subject. Several of these topics, however, have been adequately discussed elsewhere and the reader is referred in the text to these works for further study. Representative, final topics are discussed by those who are identified with the particular line of study. In respect to technology, we have attempted to present the American viewpoint and practice with greater emphasis, since this seems to have been neglected in earlier texts.

The writer desires to express his thanks and gratitude to those co-authors who have so graciously cooperated in writing the text, to his associates at the National Starch Products Refining Company for their help and constructive criticism, to his wife, Dorothy E. Kerr, for her aid in preparing the manuscript, to Dr. W. P. Pigman for reading the text and to Mrs. Lucia Dawe for reading the manuscript and the proofs. Especial thanks are due the publishers, the Academic Press, Inc., who suggested writing this timely text and who have made its publication possible.

RALPH W. KERR

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SECTION I. OCCURRENCE IN NATURE

CHAPTER I

OCCURRENCE AND VARIETIES OF STARCH

O. R. TRUBELL

1. Introduction: Source of Starch. Starch is the chief reserve carbohydrate of plants. It is therefore one of the most widely distributed substances in the vegetable kingdom. For example, it occurs in seeds and fruits as a relatively permanent reserve to be drawn upon by the young embryo when the seeds or fruits are planted. In such cases it is usually found accompanied by other reserves, such as proteins, fats, and organic phosphates. Starch is found in the vegetative parts of plants, such as tubers, the living cells of the pith, and in the cortex of roots and stems. Starch is also found in the latex of certain plants, and it may be found in transitory form in green leaves. A substance closely related to starch, particularly to some varieties of starch, is found in the liver and other tissues of higher vertebrates as a temporary reserve carbohydrate. It is called glycogen or animal starch.

In parts of plants where starch is stored as a more permanent reserve the amount present may be quite large. This is especially true of some seeds or grains, which may contain as high as 70% of starch. To a lesser extent it is true of tubers and roots and the pithy stems of certain palms, which may contain 25 to 30% of starch.

Although starches from other sources have an academic interest in the rounding out of a study of the problems involved in starch chemistry, only those which can be obtained in high yield from plants which grow or are cultivated in abundance, such as cereal grains, roots, and tubers, are of industrial importance at present. The work reported here is concerned chiefly with a discussion of these latter varieties.

Starch granules from different sources show much variety in shape and size and other superficial physical characteristics. Microscopic examination furnishes considerable information not only for the determination of the origin or variety of an unknown sample but also on given types of starch, which may aid in a fuller understanding of its behavior.

2. Size of Starch Granules. Starches from different sources show a wide range in average size. Starch granules from canna and white potato are among the largest; those from rice and buckwheat are among the smallest. While it has long been known that the larger granules of any particular type of starch, such as corn, gelatinize more easily than the smaller granules (1), it would now

appear that there may be some correlation between the dispersibility of a type of starch and its average granule size. Pacsu (2) has arranged a series of starches according to average granule size and reports that not only does the heat of gelatinization decrease with average granule size for the series consisting of potato, tapioca, wheat, corn, and rice, but also that the maximum consistency of paste obtainable in pyridine-water mixtures falls off with decrease in granule size.

Generally, the size of starch granules is expressed as the length of the longest axis, in microns. The sizes of the largest and smallest granules are noted, and the result for a given sample expressed either as a range or as an average size. Some starches, such as canna or rice, show granules which differ little in size from the mean. In these cases the average granule size is a significant value. Other starches, such as maize, show quite a range in granule size, while still others, rye and wheat starches, are composed partly of relatively large and partly of very small granules. Starch granules vary in size from about $2\ \mu$ to $150\ \mu$.

3. Shape of Starch Granules. Of less significance is the actual shape of starch. From an examination of the variety of shapes from any one type, as for example corn starch, it is apparent that the final shape depends to a large extent on the environment surrounding the growing starch granule. Those of corn starch, which are laid down in a glutinous matrix, so to speak, become compressed as the grain matures and the gluten dehydrates. The result is a horny variety of starch, very angular in shape and highly refractive (Fig. 13). At the other extreme, starch which is deposited in the crown of the corn kernel is very loosely packed and easily separated out by mechanical means. This type is referred to as floury and under the microscope appears to be perfectly round (Fig. 14). These latter granules are relatively fragile in comparison with the horny type and are easily injured during milling.

In general, starches which are formed under continuously higher moisture conditions and in the absence of horny, glutinous substances tend to be larger, more regular in shape, and more fragile. Examples are the canna, the potato, tapioca, and arrowroot. At the other extreme we have horny corn starch, millet, and rice starch.

The descriptive terms applied to the former types are (a) round, (b) elliptical, or (c) oval; to the latter, (d) polygonal.

The polygonal shape, as intimated above, probably results from close packing. Occasionally the crowding together of granules is such that separation into individual granules is not complete in the process of milling. Such aggregates of two or more granules are referred to, although erroneously, as "compound granules." True compound granules are rare and are probably another of nature's freaks like the double yolks in eggs. Occasionally the more rounded granules grow in such proximity to each other that, before milling, compound granules may be observed. When these compound granules are separated in milling the starch, a fifth form is observed, called kettle shape or (e) truncated.

4. The Hilum. This spot on the granule, clearly discernible in some starches,

is said to be the organic center or nucleus around which the granule has grown. It may correspond with, but more frequently does not, the geometric center of the granule.

The hilum is more precisely located by observing starch granules under polarized light. One theory of granule composition suggests that the density and distribution of moisture throughout the granule are not uniform. In the growing granule the hilum contains more water and is softer than other regions. As the granules dry out, stresses develop within the stratified structure of the granule, which results in the latter becoming brilliantly illuminated in polarized light, with the exception of two dark lines which intersect at the hilum. Normally these lines form a cross (Fig. 16), but as the hilum tends to be more eccentric the X-shaped cross approaches the form of a V (Fig. 17). As granules tend to become overdry, or take on excessive water, as in gelatinization, the refraction is lost; this is evidence that pressures have been relieved or equalized. In the former case the refraction of the granules may be increased or restored by re-moistening.

These facts have been interpreted differently in recent years. X-ray powder diffraction patterns of starch suggest a crystalline lattice within the granule. Since these patterns change with moisture relationships, it would seem that the elements of water enter into the crystalline structure. Anisotropy may therefore be due to a crystal-like structure within the granule.

Whatever the ultimate explanation may be, it would seem that as some starch granules dehydrate during maturity or the drying operation in normal starch manufacture this dehydration is of sufficient non-uniformity so that cracks appear on the surface of the granule. These are visible in ordinary light and seem to originate from the hilum.

The location of the hilum, by an inspection of characteristic fissures, or by observation of the darkened cross in polarized light, is helpful in characterizing the various starches.

5. Striations. By careful examination, under the microscope, some starches show a series of markings arranged concentrically around the hilum; these are called "striations." In some starches, such as that of the potato, these markings are quite plain. In others, it is necessary to stain the granules with dilute chromic acid to render them visible, while in still others there is apparently no evidence of these supposed lamellations whatsoever. These markings are best seen, when present, by oblique illumination and careful focusing. Rarely at one time are more than a few granules in the field in sufficiently sharp focus to show the striations.

It was formerly thought that the striations were significant clues to the organization of the granule. It would now seem that these markings are superficial. For example, where they are most pronounced, as in potato starch, one might expect that if sufficient force was applied to crush the granule, it might tend to fracture first along parallel lines. Figs. 30 and 31, however, show that the starch fractures most easily along radial lines. Moreover, the striations should not be

assumed to be necessarily connected with the finer, physical organization of the granule; *i.e.*, its molecular arrangement. This is discussed in the next chapter.

It may be that the number and arrangement of the striations are the result of and related to some rhythmic force or set of fluctuating conditions of botanical growth. While it has been established that these markings do not correspond to definite intervals of solar time in the growth period of the plant from which they are obtained, one of the most logical explanations for their existence is that they represent periods of relative inactivity of deposition of starchy material, either by apposition or by intussusception, as the case may be. If granule growth is by the former process, then the outermost layer is the last deposited. If growth is by intussusception, then the outermost layer is the oldest. This latter view would be more in accord with the fact that the outer layer appears to be more dense, less hydrated, and least susceptible to enzymic attack than any other region of the granule, particularly in such starches as corn starch. Ling (3) has claimed that the original, outermost layer of some starches, such as potato, is lost in milling, but may be found with the pulp residue.

Whichever is the manner of growth, as layers of dissolved constituents are deposited, followed by periods of inactivity, it is supposed that the surface of these layers either agglutinates or condenses, or continues to increase in molecular magnitude or to be modified chemically into a more impervious membrane, which latter resists re-resolution of the newly deposited granule layers. This action is possibly the result of contact with the cell sap, its hydrogen ions, metallic ions, its enzymes, or some unknown stimulus.

In some instances, a comparatively large amount of re-resolution may take place when these membranes (used in the colloidal sense) do not form. In other cases, even though they do form, their permeability may remain high and their dispersibility in water may not decrease because of a high concentration of ions in the cell sap which favors the retention of permeability.

For a detailed treatment of the subject of growth and organization of the starch granule the reader is referred to many dissertations which have appeared on the subject in recent years (4-8).

As pointed out by Badenhuizen (5), in spite of many controversial issues concerning the microscopic anatomy of starch which exist, it is, nevertheless, a noteworthy fact that three recent workers, Alsberg (4), Frey-Wyssling (8), and Badenhuizen (5, 6) have presented the same general picture.

It is unfortunate that the view presented rests largely on the concept of chemical homogeneity of starch grains in respect to carbohydrate constituents. These reviews should therefore be considered in the light of more recent knowledge of starch composition and physical properties as presented in the following chapters.

6. Swelling of Granules in Water. In the natural state, starch is insoluble in cold water but, on heating, the granules swell, rupture, and pass into a state of colloidal dispersion. The manner of swelling, as observed in the microscope, is used as another aid in distinguishing the various types of starch and occasion-

ally obtaining a clue as to the history of its manufacture. Application of this technique has been described by Sjostrom (7). Frequently, starch products are obtained in practice that are so modified in physical appearance that identification is almost impossible by usual methods of observation. These are usually specimens in which the granules are in various stages of gelatinization and are often present as mixtures of starch, such as corn mixed with wheat starch, or starch and gluten, etc. The characteristic manner in which the various granules swell being known, it is less difficult to make an identification. Details of the methods used are given by Sjostrom. Only representative examples will be given in this work, which may illustrate fundamental considerations.

Fig. 18 shows the very first stage of swelling of rye starch at 55° C. in water. This picture, taken at a magnification of 450, is, according to Sjostrom, visual confirmation of a granular organization similar to the trichite structure (Fig. 1) proposed by Meyer (9). Organization is definitely along radial and concentric lines, and it might be supposed that the microstructure of the granule would reflect its submicrostructural organization.

Figs. 19 to 26 show the early stages of swelling of potato, sweet potato, tapioca, canna, sago, and rye starches at 65–70° C., in water. According to Badenhuizen (5) swelling is tangential exclusively, the lamellae confluing finally to form the outer sac of the swollen or pasted granule. The growth of this sac is not due to internal pressure according to this worker. Rather, the internal layers are pulled asunder by the peripheral ones. This would account for the apparent free space often seen in the center of granules as they start to swell. Fig. 19 shows one such potato starch granule with an apparent free space. Swelling does not appear to be due to peripheral forces alone, and the final sac does not appear to be formed by the confluing of concentrically arranged layers of the starch. Rather the organization is both radial and concentric and smaller units form within the granule, with each of these units showing its own striations. The finer organization of these subgranule units is shown in Figs. 20 and 21 when the potato starch, before forming a paste, has been mildly treated with acid to weaken organization along concentric lines (see below).

Fig. 22 for canna starch and, more particularly, Fig. 23 for sweet potato starch show granules at the instant the internal pressure has exceeded the elastic limit of the outermost layers at some part. As pointed out by Sjostrom, the granule virtually explodes in this region. Furthermore, it is obvious, in the case of the sweet potato starch, that hydration and swelling have been retarded in one section of the granule and that it has not, therefore, proceeded uniformly in respect to the concentric layers. It should be remembered that these starches were photographed in the early stages of swelling, not the final. They show differences from some of the other starches, such as corn starch (Fig. 27). Fig. 25 illustrates the peculiar curled shape assumed by the granules, such as rye starch, in heated water above 65° C. Curling is characteristic of both wheat and rye and has been used to identify these starches in the later stages of swelling.

Some treatments used in starch manufacture, such as dry heat, weaken the

organization of the granule more along concentric lines, as noted by Sjöström (7) and Badenhuizen (5, 10). But other treatments, as with weak acid, apparently weaken the structure more along radial lines (7). Fig. 28 illustrates the initial phases of swelling of a torrefaction dextrin from corn. The gradual loosening of concentric layers is clearly shown. Fig. 29 shows the radial cracks that develop when an acid-modified, thin boiling starch is heated in water to 95° C. The acid treatment has definitely reduced the tendency of the granule to swell. Sjöström interprets this to mean that the outer layers have become less elastic and will not swell and yield to increasing pressures. The granule cracks into smaller botanical units, and a lowered paste viscosity results.

Proof that the organization which resists inhibition of water and swelling extends along radial lines is obtained by crushing starch granules with pressure. Fig. 30 shows the radial cracks that develop, extending towards the hilum, when potato starch in water is pressed between two glass plates. The effect may be exaggerated by using an acid-modified potato starch as shown in Fig. 31. Suspended in water, the units remain well defined and show practically no tendency to swell. Reference to Fig. 14 shows round and more fragile types of corn starch granules fractured, during manufacture, along radial lines but with no tendency to swell in cold water.

On the other hand if starch is ground as in a ball mill, so that eventually radial lines of cleavage are disturbed, as well as concentric, this starch will swell in cold water as shown in Fig. 32.

7. Description of Individual Starches. The following brief summary of the more common individual starches met with in industrial practice is given. Included is a discussion of several starches which, although they have not as yet assumed commercial importance except locally, are no doubt destined to be of great industrial value in replacing certain foreign starches which are not obtainable in periods of disruption of world markets. These are the sweet potato starch and the waxy starches, particularly waxy maize and waxy sorghum, which, as the following chapters show, may be used within limits when the use of a non-cereal starch such as that of sago or tapioca is indicated.

The manufacture of sweet potato starch is being developed at the present time in the southern sections of the United States under the original sponsorship of the government. Waxy maize starch has been developed into hybrids which show promise of cultivation for the industrial manufacture of starch. Leaders in this development have been the group at the Iowa State College of Agriculture and at the University of Wisconsin. The industrial application of waxy sorghum starch has been stimulated by the work at the Universities of Nebraska and Kansas.

A description of the various starches is given in the order of their industrial importance in America. This is followed by photomicrographs arranged in the customary order of granule size.

Corn Starch (Maize). Corn starch is composed of granules of two shapes, depending on whether they are derived from the crown or floury regions of

ordinary corn grains or the horny portion of the endosperm. Some species which are floury in type, such as Mandan corn, contain practically all round-shaped granules (Fig. 14). Horny species, such as Kutias, contain practically all polygonal-shaped granules (Fig. 13). Each type shows variations in size, but the average for both is about $15\ \mu$. Starch of modern manufacture will show a small proportion of very small granules of the order of $5\ \mu$. The upper limits of size are usually about 25 to $26\ \mu$.

Compound granules are extremely rare. When observed in the corn grain, the granules exhibit a large circular hilum, but in a milled, dried starch the hilum is replaced by what appears to be a cavity from which fissures radiate. Striations are absent. The polarization cross is quite distinct and appears at the hilum or geometric center.

Tapioca Starch. The granules of *tapioca* or *cassava* starch average about the same size as those of corn starch; however, they differ in appearance from the latter in that they are round or oval in shape with an indentation on one side which is characteristic of this starch. Apart from the truncated shape, with an occasional conical pit extending almost to the hilum, this starch has very few characteristic features. The granules are softer than those of corn starch, and their structure is much less rigid and compact. The hilum is centric with some fissures. No striations can be seen. In polarized light a well defined cross is observed. The size of the granules varies from 5 to $35\ \mu$ with an average of $20\ \mu$.

Potato Starch. The granules of *potato* starch vary greatly in size, from 15 to $100\ \mu$. The largest are egg-shaped and, because of pronounced concentric striations, have the appearance of oyster shells. The hilum can be distinctly seen as a dot or short line eccentrically situated in the smaller end of the granule. The polarization cross is distinct and irregular. The small granules representing early stages of development are round or oval-shaped with only faint striations or hilum.

Wheat Starch. The *wheat* starch granules, like those of rye and barley, are either large or small, with few granules of intermediate size. The granules of wheat starch are thin and of elliptical or round shape with no distinct striations. A faint *centric* hilum can be seen in the larger granules (varying from 20 to $35\ \mu$), whereas the smaller granules (from 2 to $10\ \mu$) show no hilum. By polarized light some granules show faint indistinct crosses at the center; others show no lines. Sjostrom has characterized wheat and rye starches by the observation that at lower temperatures (65 – 70°C .) the granules swell into a bag-like shape, but near the boiling point they assume a peculiar and characteristic curved shape.

Sago Starch. *Sago* starch belongs to the group of starches with a large granule. The granules vary from 20 to $60\ \mu$, are oval or egg-shaped, and resemble arrowroot and medium sized potato granules. Some appear as truncated ovals. Sjostrom has identified sago and arrowroot granules as bag-shaped with smooth outlines on gelatinization. This condition is maintained even at boiling temperatures. He has distinguished sago from arrowroot in that the former in this swollen state nearly always exhibits a well defined crater-like opening at the end of the granule.

The swollen bag-like granules of the latter starch are much more irregular in outline and the openings are large, with indefinite contours.

The granules vary in size from 15 to 65 μ with faint concentric striae on the larger and more fully developed ones. The hilum is eccentric, and the polarization lines cross it in irregular patterns.

Arrowroot Starch. The average granule size and shape of *arrowroot starch* are similar to those of sago, but vary according to the source. Some varieties show numerous truncated granules but these latter are quite possibly related to "Brazilian arrowroot" or *Manihot*. Arrowroot granules from *Maranta* roots are simple, and range in length from 15 to 70 μ . Most *Maranta* of commerce consists of granules of fairly uniform size between 25 and 50 μ in length. Striations are usually faintly concentric with a centric hilum at which distinct polarization lines intersect. The hilum is located by a characteristic, double curved fissure.

Rice Starch. The *rice starch* granules are the smallest of the starches of commerce; their size is from 3 to 8 μ . The large granules are about the same size as, but more angular than, the smaller ones of corn. Their shape is definitely polygonal and they are sometimes found honeycombed in clusters or "compound" granules consisting of numerous component granules, the outline of the whole being either round or polygonal. Because of its small size, the characteristic features of the granule such as hilum, striations, and lines by polarized light are not distinct.

Barley Starch. Generally *barley starch* is found as a mixture of large and very small granules. These large and small granules are round or elliptical and measure from 20 to 35 μ for the former and 2 to 6 μ for the latter. The hilum, fissures, and striations are indistinct even with the large granules. Under polarized light the granule usually appears uniformly illuminated, with no lines.

Waxy Sorghum (Red Leoti). The size and shape of the *waxy sorghum* starch granule, particularly that of the Red Leoti strain, are very similar to those of corn and waxy maize. The average granule size is 15 μ , with extreme sizes of 6 and 30 μ . The hilum is centric and distinct with radial fissures. Striations are indistinct. The black lines by polarized light appear as regular crosses.

This starch, like that of waxy maize, can be distinguished from common corn by the formation of a reddish brown color with iodine.

Sweet Potato. The most common form of the *sweet potato* granule is polygonal and refractive, with the predominating smaller granules appearing similar in size to those of corn (10 to 25 μ). The hilum is centric and distinct, but striations are faint, if any. The black lines by polarized light are distinct and cross at the hilum.

Waxy Maize Starch. The starch granule of the *waxy maize* hybrids now grown is similar in appearance to that of common corn with respect to size, shape, and characteristic markings. The size range is from 5 to 25 μ , with distinct centric hilum. The polarization cross is also distinct and regular. Similar to waxy sorghum, waxy maize is distinguished from corn by its reddish brown coloration with iodine.

8. Photomicrographs of Various Starches¹

Photomicrographs of various starches commonly encountered in practice are presented in the following pages, 10–17. Included also are those of the newly developed waxy starches, maize and sorghum. Photomicrographs of starches in various stages of gelatinization are also given. It should be pointed out that in all cases gelatinization was accomplished in aqueous media, with heat where specified, and not by the use of chemical swelling agents. The mechanism of gelatinization may not necessarily be the same in the two instances. Unless noted to the contrary, all photomicrographs were taken at a magnification of 200.

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¹ Figs. 1 and 16 to 32 are through the courtesy of O. Sjostrom and Industrial and Engineering Chemistry.

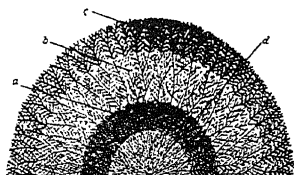


FIG. 1. Trichite structure of the starch granule according to A. Meyer.

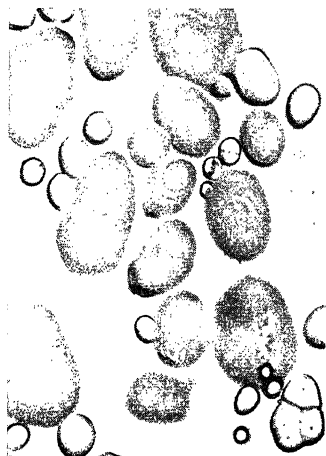


FIG. 2. Potato starch.



FIG. 3. Sago starch.

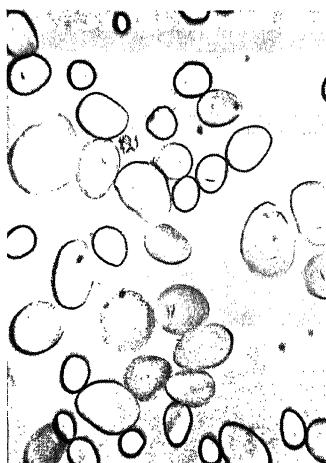


FIG. 4. Arrowroot starch.

SECTION II. PREPARATION

INTRODUCTION

The manufacture of starch and starch products may be divided into first, the preparation of starch in as nearly a native state as practical, and secondly, modifications designed to alter some physical characteristic of the native starch. The first division may be conveniently subdivided into the preparation of various botanical types of starch; *e.g.*, corn, tapioca, potato, and wheat. The second division includes a great variety of processes such as those in which the change in the starch is essentially physical, as for example, pregelatinized starch products, bleached starches, and deodorized starches. Included in the latter group are those in which, to produce the desired change in physical characteristics, an alteration in at least some of the molecules of the starch is required. Under the last named division, rather extensive modifications might logically be discussed, such as those by which the so called oxidized starches are produced. However, it is believed more desirable to consider these manufacturing processes in connection with a discussion of what is known of the chemistry involved. They will, therefore, be taken up under the general heading of "Reactions."

Previous works have dealt rather extensively with the manufacture of white potato, tapioca, sago, and other starches imported into the United States. In the Americas the manufacture of cereal starches and, in particular, corn starch is of primary importance. A description of the manufacturing processes for corn starch will be dealt with in detail. Only a brief description of the processes for the other industrial starches will be given.

The manufacture of corn starch, in common with the relatively small amount of potato starch made in America, is placed at a disadvantage, in that a raw product must be used, for economic reasons, which is cultivated with little regard to the quality of starch it contains or its suitability for milling. Both industries utilize less than 1% of the total crop for the production of starch to be sold as such. Corn, in particular, is cultivated for its hardness, its yield of crop per acre, and the protein and oil content of the grain. This has led to the custom of growing a horny type of corn in recent years, containing less of the softer, floury variety of starch, which latter is unfortunately more readily milled and separated. The cullings, which are the small, broken, misshaped, or otherwise unmarketable potatoes, are the principal raw material for making potato starch, particularly in the United States. In contrast, *Manihot* is frequently cultivated primarily for the manufacture of tapioca starch. In some cases the plantations are owned or controlled by the mills producing the starch.

The manufacture of corn starch is further complicated, when compared with tapioca starch, for example, by the fact that the sale of native starch, as such, represents but one of the outlets for the product. A considerable percentage of corn starch is immediately treated to make sirups, sugars, modified starches, and dextrans, the former two consuming more than half the corn starch made. The characteristics desired in a starch for one use may be of little importance when the starch is considered for another purpose. For example, the chief industrial use of starch, as such, depends on its property of gelatinization in hot water. An important property of such a colloidal system is viscosity, but obviously, whether a corn starch will gelatinize to a thicker or thinner paste is of minor importance, if the starch is to be hydrolyzed to dextrose.

Hence, in a comparison of the various processes to be described for corn starch with those for other starches it should be borne in mind that the former were designed to produce a superior product for many and diverse uses.

CHAPTER II

THE MANUFACTURE OF CORN STARCH *

1. The Corn Grain. From the view-point of the miller the corn grain (*Zea mays*) may be considered as divisible into four definite regions: (*a*) an outer hull which encases the other three regions, (*b*) the embryo, or germ, which is located near the tip of the grain, (*c*) that part of the endosperm which is light in color, often located at the opposite end from the germ and referred to as the crown, and (*d*) the remainder of the endosperm which is more intensely colored than other regions in yellow varieties of corn.

The hulls contain principally insoluble, non-starchy carbohydrates and some inorganic constituents. They are readily loosened and removed by soaking the grain in warm water, followed by passage through coarse mills, and then over screens.

The germ contains substantial quantities of water-soluble constituents, such as inorganic salts, soluble proteins, and, in addition, natural fats of low melting point. Owing to its oil content which is, in effect, increased in concentration after the water-soluble constituents are leached away, the specific gravity of the remaining germ is lower than other component parts of the grain. This lower specific gravity is utilized in separating it in the early stages of the milling process.

The crown regions of the endosperm consist primarily of starch which is packed and held quite loosely when compared with starch embedded in the horny regions of the endosperm. Hence, as might be anticipated, very little milling is required to release the starch held in the crown region, which starch is of prime

* The photographs are reproduced through the courtesy of the Corn Industries Research Foundation.

quality, consisting of large, less angular granules of relatively high potential paste viscosity. The amount of crown starch varies with the variety of corn. In certain varieties, known as Mandan, which are grown in the northwest and north-central portions of the United States as much as 75% of the starch content of the grain may be found in the crown, whereas in the more flinty varieties, such as hybrids of the Russian Kutias type, practically no crown starch is in evidence.

The remainder of the endosperm consists essentially of small, polygonal starch grains embedded in layers of a relatively water-insoluble protein. These layers are rendered more water-impervious by the small amounts of oil and carotenoid substances which are associated with them. The proteins present in this region are classed as gluten and glutenin and are referred to as zein and zeinin, respectively. Although relatively water-insoluble, they do swell and to some extent dissolve in warm, acidulated water. Because of this fact, grain which has been thoroughly soaked in water acidulated with sulfurous acid (which water contains substantial amounts of lactic acid that normally develop during a 36 to 48 hr. soaking period) may be milled by fine grinding to loosen all but a small portion of the starch it contains. Further treatment of the residual slurries with dilute sodium hydroxide, in which the zein and zeinin are also partially soluble, will facilitate the removal of the balance of the starch from the protein with which it is associated.

Although the intensity of milling operations would quite obviously be greatly varied to process the two extreme varieties of corn grain mentioned (Mandan and Kutias), nevertheless the varieties of corn grain most commonly processed (which possess a more even distribution between soft, crown starch and tough, horny starch) are milled in systems in which the intensity of treatment is stepped up to recover most of the starch embedded in the glutinous portions of the endosperm. It is to be expected that the less resistant crown starch suffers accordingly during these steps of starch manufacture.

2. Preliminary Treatment: Cleaning and Steeping. Practically all corn starch manufactured at the present time is milled from yellow corn bought on the open market. The corn, shelled from the cob, is delivered to the miller by rail in box cars. The grains are shoveled by power shovels onto conveyers which either carry them to storage or directly to the initial phases of the milling process. The grains are put through a cleaning system first, which involves the use of air blasts to remove dirt, dust, and chaf, and then over screens to remove stray cobs and other extraneous material.

Wet milling is the common practice. For this purpose the corn grains are soaked in warm water for various periods of time and at various temperatures, depending on the nature of the average sample, whether it is, for example, newly arrived from the fields and is of the current year's crop, or whether it has been in storage or in grain elevators. Usually the moisture content of the grain is a fair index of age and past history for any one type of corn. Its specific gravity at a given moisture content is a fair index of its "hornyness."

The soaking, or steeping, as it is more commonly called, is to facilitate separating the various components of the grain in the subsequent milling operations with the highest degree of efficiency. Hence, the importance of this primary phase cannot be overemphasized and will be gone into in some detail.

Various millers have different conceptions of the manner in which this operation should be performed. The simplest method, known as single phase steeping, consists of putting the corn into a large wooden vat, covering the grain with warm water acidulated with a small amount of sulfur dioxide to inhibit the development of microorganisms, and allowing the corn to soak until it is sufficiently soft for most of the starch to be milled out (1, 2).

One obvious disadvantage in such a system is the quantity of water required. It appears necessary to remove some water-soluble material from corn in the steeping process. This is particularly true in respect to the efficiency with which the germ is separated in the subsequent grinding and levitation procedures. To extract a given amount of soluble components, less water is required in a counter-current system (3). However, an inspection of the elementary method described is instructive in that it discloses certain advantages over other systems to be discussed. Other variables being the same, it will be found that (a) single phase steeping requires a minimum addition of antiseptic to inhibit the activity of microorganisms, and (b) by the proper balance of steeping time and temperature a starch of relatively high viscosity is obtainable.

TABLE I
Characteristics of Single Phase Steeping at 125° F. with SO₂

Steeping time	Steeping liquor				Steeped corn (200 g.)			
	Volume	pH	SO ₂	Titrateable acidity	Moisture	pH	SO ₂	Soluble
<i>hrs.</i>	<i>cc.</i>		<i>per cent</i>	<i>N</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
0	300	3.7	0.150	0.072	10.62	6.3	0	6.90
6	215	4.1	0.081	0.074	36.90	5.5	0.110	5.44
16	185	4.4	0.069	0.072	42.06	5.5	0.115	5.95
24	180	4.5	0.072	0.081	44.48	5.4	0.110	5.18
40	177	4.2	0.056	0.140	43.80	4.4	0.095	4.56

Table I gives the results of a single phase steeping experiment. 200 g. of an Illinois utility type corn were covered with 300 cc. of gluten overflow water (*q.v.*) containing 880 grains of soluble corn solids per gallon and 0.15% SO₂ was added. The glass flask was stoppered with cotton, warmed to 125° F., and held at this temperature for 6 hrs. The water was then drained from the corn and the determinations made, as reported in Table I. Exact duplicates were steeped 16, 24, and 40 hrs.

It will be observed that in such a system the concentration of antiseptic is highest at the point of entry of microorganisms into the system, both in respect

to the corn and to the process water. In spite of the fact that the acid antiseptic is rapidly absorbed by the corn, the *pH* of the latter, originally 6.3, is not materially depressed. In fact, it is only during the last hours of steeping, when the titratable acidity of the steeping medium abruptly increases, that the average internal *pH* of the corn is depressed to a level at which acid activity might be sufficient to induce hydrolysis of starch at 125° F. The Scott test (see Chapter VI) of starch milled from corn as steeped above averages between 115 and 120.

Although a ratio of nearly 11 gals. of steeping liquor has been used per bushel of corn, the total material soluble in cold water remaining in the corn is rather high, being over 4.5%.

The conservation of water demanded in modern practice and the prohibition of emptying process waters into sewers in most industrial sections have led, therefore, to the use of counter-current flow (4-7). One of the simplest forms of the latter is to use two or more, possibly as high as twelve, open wooden vats in a series arrangement. After the corn is steeped a given number of hours in one steep, the water is transferred to a fresh lot of corn in the next steep, and a more dilute water is placed on the corn already partially steeped. When all steeps have been put into operation, progressively, in the manner described above, the most dilute process water is placed on corn that has been steeped the longest, after which final period the softened corn is sent to the mills. Simultaneously an empty steep is being filled with dry corn which is then covered with the oldest water in the steeping system, and in turn the water in the entire system is then "advanced" in the direction of the corn last entering the system. The water, after passing through all the steeps in turn, is finally drawn off the steep last filled with corn and is known as steepwater. It is usually transferred to evaporators and concentrated, after which it is mixed with other grain components to make a feed for cattle.

The water usually used for steeping in counter-current fashion is that with the highest content of soluble material obtained from the mill house. This soluble material may average from 400 to 1000 grains per gallon. It is customary to run the water over a sulfur tower in which the correct percentage of sulfur dioxide is taken up, or else to add other germicides at this point, then heat the water to steeping temperature before placing it on the corn in the steeping system. Temperatures are usually maintained at about 125° F. at each steep by means of pumps connected to heaters. These pumps also maintain a circulation of water within each individual steep during its steeping cycle.

The water leaving the steeps usually amounts to 5 to 7 gals. per bushel of corn steeped and contains as high as 6% of non-volatile solids. The corn leaving such a system will be found to contain approximately 3% of cold water-soluble material.

The water entering such a steeping system is quite acid, possibly as low as *pH* 3.5, due to the sulfurous acid it contains, which, in more common practice, corresponds to about 0.15 to 0.20% SO₂. Few mills today use as high as 0.3% SO₂, which was the reported practice a decade or two ago. The water leaving

enzymes tend to degrade the starch or other components of the grain but is actually essential for the most efficient separation of starch from the gluten matrix in which it is laid down. These conclusions would seem to follow from the poor results obtained in the laboratory on steeping experiments with tap or distilled water as the steeping medium in a single phase steeping operation. The result is not as conclusive, however, from practical steeping experiments in which process waters are used for steeping in counter-current flow. It is *more* obvious that a certain amount of *acidity* is essential to separate starch from gluten efficiently, and it would appear to make little difference whether this acidity is added in the form of H_2SO_3 or other weak acid, or whether it is permitted to form by fermentation during the steeping cycle. It would seem that a regulated fermentation in which soluble carbohydrates are converted to lactic acid was a desirable steeping practice provided protein degradations can be kept at a minimum at the same time. Higher steeping temperatures, for example 125–130° F., appear to favor the desirable features of such a fermentation. Lower temperatures are more suited to the development of putrefactive reactions and alcoholic fermentations.

Milling and separation operations, after steeping, are usually performed at temperatures under 100° F. Hence, if a regulated bacterial activity is permitted in the steeps, then it is obvious that a pasteurization step for the corn is desirable before the corn is passed onto the mills.¹

Regulated fermentation is more desirable than a high degree of sterility in steeping for several reasons in addition to those mentioned. Conditions resulting in a high degree of sterility are apparently adverse for other enzymic reactions which may be desirable. These latter are the action of phytase and proteolytic enzymes which can convert soluble native protein as far as proteoses and peptones. The final separation of starch from gluten is facilitated by the presence of phosphate ion. A logical source of the desired phosphate would be the reserve phosphorus of the grain itself, phytin, by a phytase hydrolysis.

If steepwater is evaporated from a counter-current steeping system in which process water is used for steeping and a high degree of sterility has been maintained in the system, considerable difficulty may be experienced in evaporating the water owing to coagulation and precipitation of certain soluble material which forms a deposit, or scale, on the heating surfaces of the evaporators. So serious has this condition been in the past that when the disposal of steepwater into sewers was prohibited, the capacity of plants milling corn starch has been restricted by the amount of steepwater they could evaporate. The scale formed consists principally of salts of calcium and magnesium and smaller amounts of native or coagulable protein which are solubilized during steeping by the acid

¹ For a discussion of the flora that develops from corn grain in wet milling and a discussion of its control with sulfur dioxide, one is referred to the dissertation of Killinger (23). In this connection it is to be noted that a patent was granted to Acton (24) for the control of fermentation in milling, a balance of pH being used against the temperature of the liquid phase, which claims are in harmony with the results found by Killinger.

steeping medium. A regulated fermentation will provide a sufficient non-volatile acidity, such as lactic acid, which will maintain the solubility of the alkaline earths during evaporation of the water. This fermentation induces simultaneously the degradation of soluble protein by enzymic or bacterial activity into constituents which do not coagulate on heating.

For systems badly out of balance, corrective measures may be applied to the water after steeping by drawing the steepwater into holding tanks and then allowing the desired lactic acid fermentation of the steepwater to take place (25). Two more recently issued patents (26) for the pretreatment of steepwater involve a short pressure-cooking which probably permanently solubilizes some of the native protein dissolved in steepwaters containing an excessive proportion of this constituent by a slight hydrolysis and precoagulates a part of the remainder. However, it would seem that no provision had been made in either of the newer methods to overcome scale formation in the evaporator from lime and magnesium salts, which are solubilized by acid steeping waters during steeping. As the volatile acidity of the water drawn is removed by vacuum evaporation, the acid salts tend to change to neutral salts which are relatively insoluble and form crusts on the steam tubes, steam chest, and other heating surfaces of the evaporator. Hence, this method would appear applicable only to water from special steeping systems; for example, those in which lactic or other non-volatile acid fermentation is permitted, or, possibly, to alkaline steeping systems. The older method of treatment (25) is not limited in its use to any particular steeping method and has proved satisfactory in conjunction with systems run on a high level of sterility, as well as those in which no antiseptic is used whatsoever.

Finally, in this connection, in one system of steeping the cultivation and propagation of desirable lactic acid bacteria are actually encouraged. The author has supervised the use of such a system in plant practice for several months to prove that germicides and, in particular, SO_2 could be dispensed with in the actual steeping operation.

Aside from the concentration of antiseptic and the amount of steepwater drawn off in steeping, the main variables of the steeping process are time, temperature, and pH. The last three variables are the same which, if maladjusted, induce an acid hydrolysis of the starch. The first apparent effect of this reaction is a decided loss in the potential hot paste viscosity of the starch.

The effective pH in this action is not that of the steeping media necessarily, but rather the pH of the moisture or water layers inside the kernel, which are in immediate contact with the starch granule. Actually, there may be a wide difference between the two acidities. The neglect of this fact may explain why attempts to buffer the steeping medium with sodium carbonate frequently do not give all the beneficial results anticipated. In addition, this unnecessary corrective measure induces certain undesirable actions. As sodium carbonate or other alkali is added to the counter-current systems described above and the pH is thereby raised, the germicidal efficiency of sulfurous acid is materially decreased. Fermentations become more vigorous with the result that either an

increased amount of SO_2 must be added or lactic acid production is materially stepped-up. Both results lead to a regain in the acid concentration of the system, and it then becomes necessary to add further quantities of sodium carbonate to buffer this new increase in acidity. This vicious cycle repeats itself and very soon it is found that unbelievably large quantities of alkali are required to fix the $p\text{H}$ of the steeping medium. The alternative is to add sufficient alkali directly, so that a $p\text{H}$ is maintained far enough on the alkaline side that fermentations are restricted. Alkaline steeping has, however, passed out of vogue because of certain shortcomings that are not found in acid systems.

A more logical solution of the problem, to protect the starch against hydrolytic action during steeping, is to make better use of the natural buffers that exist within the corn kernel. Although the larger part of these is found in the germ, nevertheless this locality is quite likely the point of entry of the steeping water and the ions it contains into the interior of the corn kernel.

These buffers are not readily soluble in their original form, and some are not diffusible through the partitioning membranes of the grain. Hence, the concentration of hydrogen ions invading the kernel in the early stages of steeping is quickly depressed when these ions enter the kernel and are contacted by these buffers. The difference existing between the average internal $p\text{H}$ and external $p\text{H}$ in steeping with counter-current flow in open vats is illustrated in the results listed in Table II. Twelve steps were used in series in this experiment and process water containing 600 grains per gallon of soluble material, 0.17% SO_2 , and having a $p\text{H}$ of 3.5 to 3.7 was added to the system for steeping. About 6 gals. of steepwater at $p\text{H}$ 4.2 with an SO_2 concentration of 0.04% were being drawn from the system for each original bushel of corn steeped.

At a given time samples of corn from the interior of each steep in series were removed, with a sampling device, and immediately rinsed with ice water. The samples were finely ground and shaken intermittently with cold distilled water for about 30 min. The extracts were filtered and a $p\text{H}$ determination on each was made. At the same time that the corn samples were taken, samples of the steeping media were secured at each steep and a $p\text{H}$ determination on each was made. The steeping "cycle" was about 46 hrs. However, the actual steeping time was probably 40 hrs. or less owing to the time required to fill and empty steeps with corn and to advance the steeping liquor by pump in large scale operations.

In steeping, most of the corn grain buffers soon become solubilized by the invading acid and they tend to diffuse into the steeping media, raising the $p\text{H}$ of the latter. In counter-current steeping, moreover, these extracted buffers are soon removed from the steep in which they were extracted, and a new lot of water, more acid in nature, is added to leach out additional quantities of buffer. The result is that for more than 50% of the total steeping time the starch is in contact with an acidity which at 125° F. induces a pronounced degradation of the starch. The hot paste viscosity of the starch, as measured by the Scott test (a specific viscosity test used in practice), will be observed to drop during

this steeping cycle from about 125 (starch from Steep 2) to about 85 (starch from Steep 14). Such a marked drop in the average internal pH of the corn is not observed in a comparable single phase steeping system in spite of the fact that a more acid water, pH 3.5, is placed immediately on the dry corn and that whatever sulfurous acid is going to penetrate the kernal is known to do so in the first 6 hrs. Furthermore, in such single phase steeping the average internal pH of corn falls only to about 5.4 in the first 24 hrs. and then gradually drops off to pH 4.4 in the next 16 hrs. The Scott viscosity value of the starch from such a system as described has been found to average 115.

It is apparent, therefore, that excessive leaching out of the natural buffers of the grain as in counter-current steeping is to be avoided in steeping if a starch of high viscosity is to be obtained in acid steeping processes.

Various corrective measures have been proposed to prevent such action. The most elementary proposal is to reduce the amount of steepwater drawn. This remedy results in a decided increase in the soluble solids of the process waters throughout the milling and separating operations which, for certain reasons, is not desirable. Another proposal is to introduce a second steeping phase after the corn has been milled. This, so far as is known, has not proved to be practical. Another elementary proposal is to return a portion of the steepwater drawn to the steeping system. The objections to this procedure are obvious.

All of these measures, however, disregard the fundamental fault of counter-current steeping, in that it more effectively and more quickly removes the mobile and permeable electrolytes from the regions where they could be effective. A system which seeks to retain the advantages of single phase steeping in these respects and at the same time results in a water economy consistent with practice, is outlined as follows: Steeping is accomplished in three phases as is indicated diagrammatically in Fig. 33. These consist of two actual steeping periods, followed by a final wash. Water from the gluten settlers plus antiseptic is used for the final washing phase. After this wash, the water is used directly for the second phase of steeping. After this phase, a small portion of the water is sent to a storage tank supplying the steepwater evaporators. The remainder of the water is mixed with sufficient gluten settler water plus antiseptic to provide for the first phase of steeping. The steepwater from the first phase of steeping is likewise sent to tanks supplying the steepwater evaporators. The temperatures maintained are those customary in steeping.

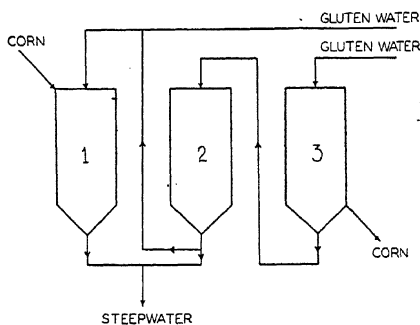


FIG. 33. Three phase steeping system

The steeping conditions used will vary and depend on the nature of the corn steeped, whether it is a new or old crop, high or low in moisture content, horny or floury, and other such variables. However, by the use of gluten settler water with a relatively high content of soluble solids, the per cent soluble of the steeped corn can be kept to a satisfactory range for high buffer action, about 3.75%, by a proper balance in the steeping variables. The result will be that, except during the last hour of washing, the internal *pH* of corn will not be depressed below 4.5. Hence, hydrolytic action on the starch will be kept to a minimum.

This steeping system is not limited in use to any particular antiseptic or sterilizing agent. It may be employed with the more commonly used reagent, SO_2 , or it may be used with the newer antiseptics, such as, for example, those suggested by Berquist (18), or a combination of reagents. One principal advantage of the system is that for a given amount of sterilizing agent used a lower level of biological activity results than when the older methods of counter-current flow are used.

By this system there is produced a steeped corn with a starch of high potential paste viscosity, well over 100 according to the Scott test.

3. Milling and Separation. The steps in the manufacture of corn starch which immediately follow steeping are essentially of a physical nature. The softened corn is ground, and constituents other than starch are progressively removed until finally only starch granules and gluten remain to be separated.

The steeped corn passes directly to coarse mills. Usually these consist of two large steel discs provided with large metal teeth which tear the grains apart into its major constituents, hull, germ, and endosperm. The discs stand in an upright position and are called Fuss mills (Figs. 34 and 35).

The grist is carried away with process water to germ separators. The water from some later stages of operation (germ reels and grit shakers) contains sufficient starch, together with the crown starch which is liberated into suspension in the Fuss mills, to raise the specific gravity to the point at which the germs will float to the surface, whereas other constituents remain suspended or sink to the bottom. Germ separators (Fig. 36) are large V-shaped vats about 4 ft. in width, 6 ft. in height, and about 10 to 12 ft. long. The grist enters at the top of one end and moves slowly to the other, where the germ is floated off at the top and the residue is drawn off at the bottom. In the meantime paddles, the width of the separator, move along the surface, aiding the movement of the floating germs, in a skimming action.

The residue passes to copper sieves of coarse mesh. These are a rotating type and as such are referred to, in general, as reels. Here the hulls are retained within the rotating sieves and are discharged at the end opposite which they enter. The finer grits and starch pass through the meshes. The coarse material, containing the hulls, passes to a second set of Fuss mills, after which it is suspended again in the liquors from the first grinding reels. This grist now passes to another series of germ separators to recover any germs escaping separation in the first separators.

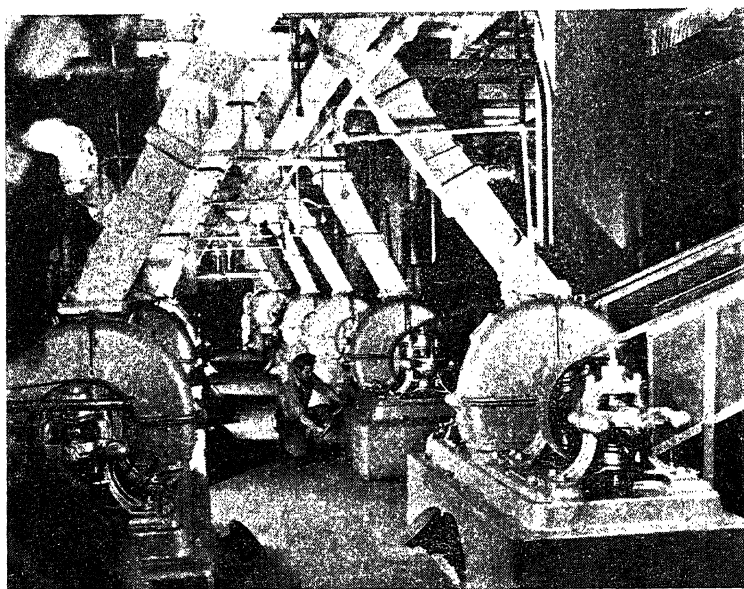


FIG. 34. Fuss mills

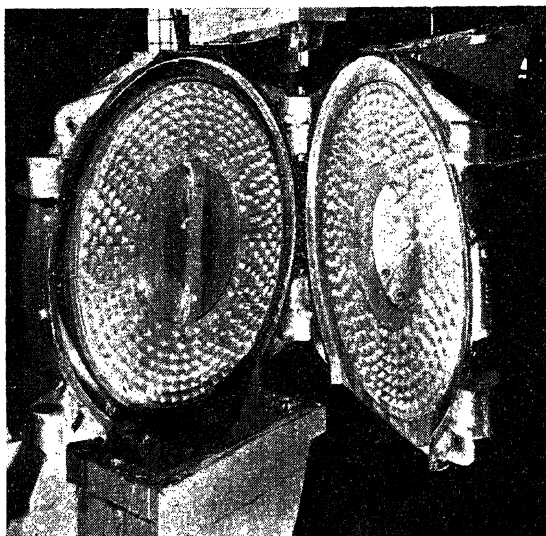


FIG. 35. Fuss mills; opened to show metal grinding surfaces

The germ is then collected and passed over a battery of reels provided with a mesh fine enough to retain the germ. Water sprays wash away the adhering



FIG. 36. Germ separators

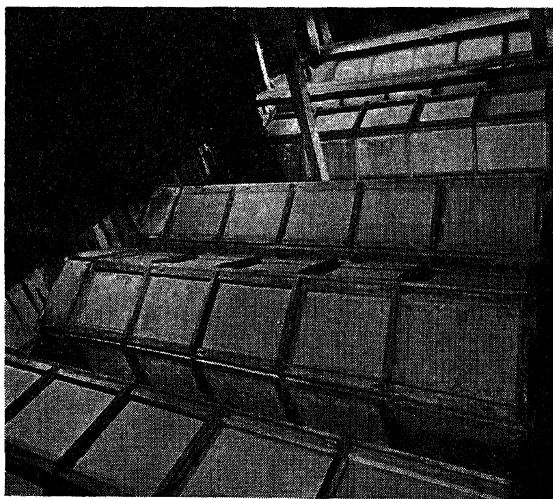


FIG. 37. Battery of reels

starch, and the wash water is returned to the milling process in the manner indicated above. The germs are dehydrated and the corn oil extracted, to be sold as a crude, or refined, oil.

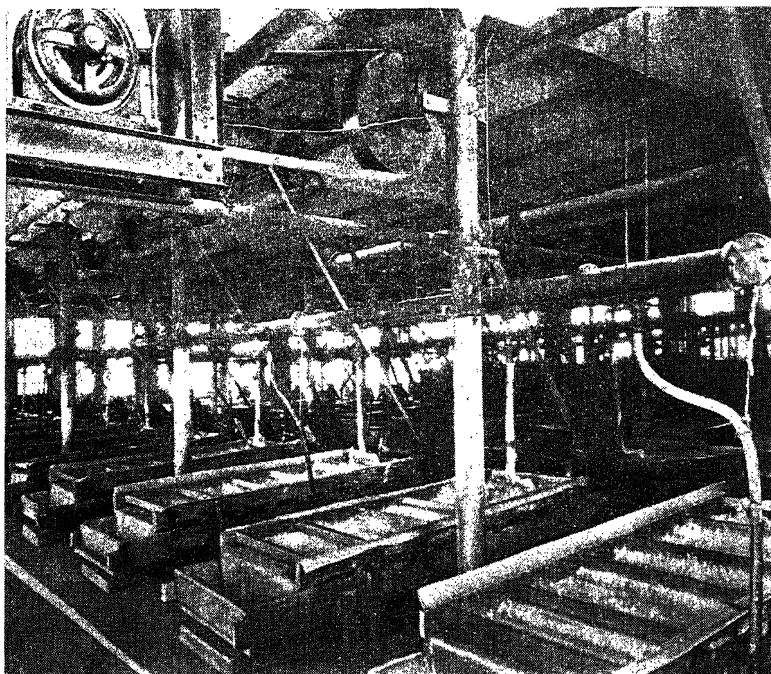


FIG. 38. Shakers

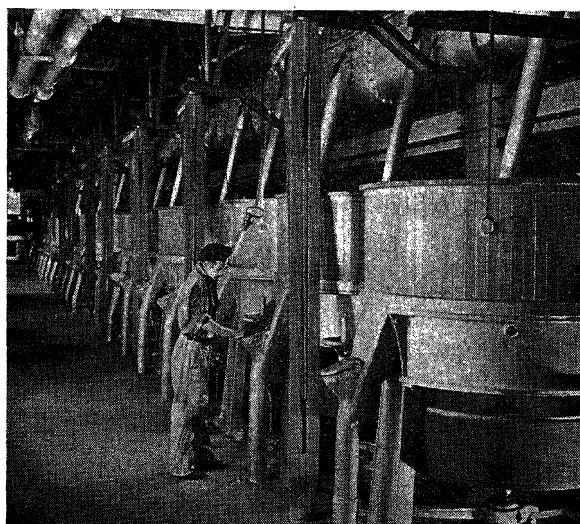


FIG. 39. Buhr mills

The residual slurry from the germ separators passes over reels to remove the hulls and other coarse particles (Fig. 37), and then over silk sieves to remove finer particles of fiber. The silk sieves are in a horizontal or slightly inclined position, and passage of the liquors containing the starch is facilitated by a vibrating motion induced by connecting the sieve frame to an eccentric. These sieves are consequently called shakers (Fig. 38).

The starch-bearing liquors pass mainly to the system supplying the starch tables or other separating equipment.

The hulls and fiber, together with coarse starch-bearing gluten particles, are collected and passed on to Buhr mills for fine grinding. These consist of two large stones, one on top of the other. The lower one is usually stationary, the upper one is rotated to produce the grinding action (Figs. 39 and 40). From this grist the coarser fiber is removed by passage over reels. The fiber is then

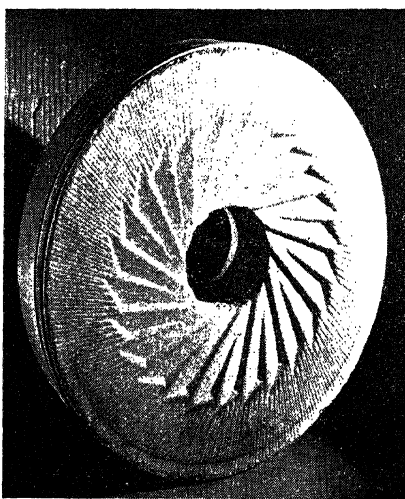


FIG. 40. Stone grinding surface of a Buhr mill

washed with water to remove the last of the free starch and is then dehydrated. After removal of the coarse fiber the slurry is passed over silk covered shakers to remove the last amounts of fiber, which latter is washed to remove any free starch and is then dehydrated.

It is essential that the last traces of fiber be removed from the starch; otherwise imperfect separation of starch from gluten is likely to result.

The starch slurries are now adjusted to the proper density for separating the starch from the gluten.

Two systems are commonly used; the older method consists of running the slurry down a slightly inclined wooden (27) or lined trough (28, 29) called a table (Fig. 41), and the

newer method involves centrifugal separation. Factors involved in tabling are more generally understood, and no doubt will be found to influence centrifugal separation to some extent as well.

It should be pointed out first of all that the cardinal requisite for good tabling, that is, efficient separation of starch from gluten, is perfection in equipment. The tables should have a smooth surface free from ridges or bumps and inclined at the proper angle or slope (30). The latter will be determined by the width, the gallons per minute flow per linear foot of table, and the specific gravity of the slurry. All of these factors are mutually dependent, one on the other, and no exact specifications can be given to meet all ranges of variation in these factors.

The table should not be so wide that a zig-zag course is permitted (31).

Otherwise, the actual flow in gallons per minute per foot of table will be different than might be anticipated. If the rate of flow is too slow, some gluten will settle out with the starch on the lower ends of the table. If the rate of flow is too fast, starch will pass off with the gluten. The correct rate of flow, once determined for a table and a given type of starch slurry, should be held absolutely constant (32, 33). There is only one such rate of flow which will give a peak efficiency in separating starch from gluten, and the permissible variation from this rate is very small indeed.

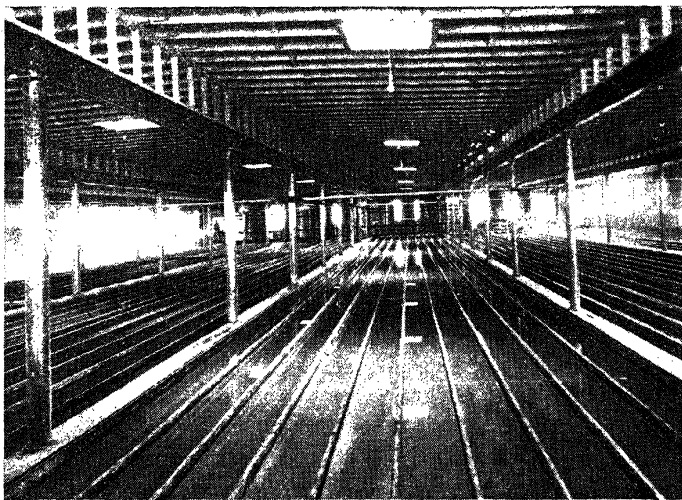


FIG. 41. Starch tables

Other factors being equal, the lower the density of the starch slurry, the easier it is to separate by tabling. In the early days of starch manufacture, 3° Bé. for table liquor was deemed to be the highest density which could be used. With a better understanding of the mechanics of tabling, this value was raised to 6° Bé. and with a fuller appreciation of the physical chemistry involved it has been found possible to double this value again to 12° Bé.

It is to be expected, then, that higher temperatures facilitate tabling in that a reduction in apparent specific gravity results. This is true as long as the temperature is not raised to a range where an undue softening or swelling action on the gluten or starch results.

There is also a pH range for table liquors which is conducive to peak separation efficiency. This is between pH 3.8 and 4.2. But inasmuch as this corresponds to the range in which sieving of the liquors, in preceding operations, will be found to work best, it is rarely ever necessary to adjust the pH at this point. Most systems of steeping used at present condition the grain so that when it is ground

up with process water in the mill house the pH of the slurries will be found to fall in the range desired, with very little readjustment. Usually the latter is accomplished by the addition of small amounts of sulfurous acid at various points, as, for example, in the coarse reels following the second Fuss mills, the coarse fiber reels following the Buhr mills, and the fine fiber reels which supply the tables or other gluten-separating devices. These additions of sulfurous acid also tend to maintain the SO_2 concentration of the liquors at a level which will inhibit the growth of undesirable bacteria and fungi. However, when non-acidic germicides are used, hydrochloric or some other suitable acid may be added at one or more of the points mentioned above.

Other things being equal, the presence of small quantities of dissolved electrolytes facilitates sedimentation of the starch. The writer has observed that phosphate, which is normally present in liquors from corn grain, is very effective. However, many electrolytes can be used, even an acid or alkali, provided a sufficient quantity is present.² Hence, we have the odd result that, whereas starch is most readily separated from gluten at a pH range of 3.8 to 4.2 (provided a total electrolyte concentration is present in the water phase of from 50 to 150 grains per gallon), as the pH is lowered, to let us say 3.0, poor sedimentation of starch from gluten results. But, nevertheless, if more of a strong acid is added to reduce the pH to 1.5, for example, fairly good separation is again obtained; likewise with the addition of alkali as we proceed into the alkaline ranges. It has been noted by the writer that although the sedimentation of starch is influenced by electrolyte concentration, the effect of the latter becomes less pronounced in table separation the lighter the density of these liquors, and more pronounced the heavier the density. For example, the effect of electrolyte is much more pronounced at 12–13° Bé. than at 10° Bé. It has also been noted that one electrolyte, at least, exerts an adverse effect on separation. This is the lactate ion or lactic acid. Inasmuch as this substance is likely to be present in wet starch milling liquors, control of its concentration is, therefore, indicated. Some of the effects given above will be illustrated by experimental results. First, however, mention must be made of another variable, very little understood, but which none the less is extremely important.

No amount of adjustment of factors described above will wholly compensate for failure to bring the gluten to the proper physical state for separation. For want of an accurate description of this physical state, it might be termed "proper degree of hydration." At present there is no precise measurement of this state. It is only noted when one attempts to table starch and gluten suspensions. If, for example, corn grain which has not been sufficiently soaked in the steeping process is milled and the table liquors are brought to a certain density and temperature, and the pH and electrolyte concentration are adjusted to optimum ranges, a fair degree of separation may be obtained. If, however, a portion of the same starch liquor is properly soaked before milling, it will table better under

² In this connection see the experiments of Wiegel and Schüler (34) on the sedimentation velocity of potato starch.

the same control of the other variables. Moreover, a change in these variables, for example in the electrolyte concentration and pH, will exert a less detrimental effect than a corresponding change of the same variables with the under-steeped corn sample.

The following section describes experiments to illustrate some of the salient points mentioned above.

Normal starch table liquor, sometimes referred to as mill starch, was obtained from an industrial process by a milling procedure essentially as given above. This suspension was filtered, resuspended in distilled water, and refiltered. The washing procedure was repeated until the water phase of the suspension showed the characteristics given in Table III. Additives, such as sodium phosphate and lactic acid, were included and water added to dilute the suspension to various densities. Unless otherwise indicated, the table liquors were then warmed to 86–88° F. and tabled at this temperature. Densities are expressed as degrees Bé., corrected to 60° F.

Two liters of liquor were tabled on an experimental starch table 10 ft. long and 3 in. wide, with an elevation of 1/16 in. per foot. A constant flow was maintained by passing the liquors at a constant head through a fixed orifice, which delivered the 2 liters in 23 min. at 12° Bé. The liquors were kept in a state of continual agitation prior to passage through the orifice.

All solids running off the tables for 31 min. after the start of tabling with liquors at 10° Bé. and higher, and for 28 min. with those at less than 10°, were taken as gluten. The starch on the tables was then washed with 800 cc. of filtered liquor, identical in each case to the liquid phase used in the tabling experiment. The table was thereupon elevated $\frac{1}{2}$ in. to the foot and 500 cc. of distilled water were delivered through the same orifice. After a 5 min. draining period the starch was removed and washed free of soluble substances. Owing to limited washing of the starch on the tables, the protein in starch is higher than would be obtained in mill practice. However, more intensive washing gave erratic results which could not be reproduced.

The acidity of the filtered mill liquor given is expressed both as pH and as titratable acidity, the latter estimated as normal acidity. Mill liquor reduced to 0.006 *N* acidity contained approximately 10 grains per gallon of soluble ash, normally. The results are given in Table III.

Experiment 1 shows tabling results obtained with a normal mill liquor at 12.8° Bé. and 0.0355 *N* acidity, whereas Experiment 2 shows the very much less efficient separation obtained by washing out soluble electrolyte to reduce the acidity to 0.0083 *N*. Experiments 3 to 6 show similar effects but which diminish in magnitude as the density of the mill liquors is reduced to 12.2° and 9.7° Bé. respectively.

Experiments 7 to 21 show the effects of adding various quantities of (normal acidity) sodium dihydrogen phosphate per 2 liters of mill liquor to mill starch, the latter being prewashed to reduce the original, soluble electrolyte concentration. Again it will be seen that efficiency in table separation increases with

TABLE III
Influence of Soluble Material on Efficiency of Table Separation

Experiment No.	Variables	Titratable acidity	pH	Density	Gluten fraction			Starch fraction	
					Yield	Starch	Protein	Yield	Protein
		N		°Bé.	g.	per cent	per cent	g.	per cent
1	Washed	0.0355	3.9	12.8	40.5	36.5	52.4	399.2	0.56
2		0.0083	3.9	12.8	35.3	47.2	43.7	392.2	1.11
3	Washed	0.0210	3.9	12.2	36.9	30.1	55.6	426.2	1.22
4		0.0053	3.9	12.2	28.5	40.2	47.4	411.4	2.34
5	Washed	0.0195	3.9	9.7	37.6	30.2	55.4	329.9	0.67
6		0.0072	3.9	9.7	26.0	35.0	51.2	340.2	1.55

Phosphate added (normal acid solution)

7	Washed	0.0069	3.8	13.2	28.4	50.8	39.5	444.9	2.56
8	" + 15 cc.	0.0153	3.8	13.3	33.6	39.6	49.0	456.5	1.82
9	" + 30 "	0.0225	3.9	13.5	34.9	30.3	56.7	455.7	1.47
10	" + 45 "	0.0317	3.9	13.7	36.0	30.7	56.7	456.8	1.14
11	Washed	0.0050	3.8	10.1	23.0	39.8	49.5	327.1	1.15
12	" + 15 cc.	0.0132	3.8	10.2	28.0	34.1	54.4	338.6	0.60
13	" + 30 "	0.0223	3.9	10.2	29.5	29.2	56.9	337.9	0.51
14	" + 45 "	0.0312	4.0	10.4	30.4	28.6	57.4	339.0	0.39
15	Washed	0.0042	3.8	8.0	19.3	34.2	51.7	254.0	1.11
16	" + 15 cc.	0.0120	3.9	8.1	24.2	30.0	55.3	265.6	0.51
17	" + 30 "	0.0200	4.0	8.1	26.3	27.9	57.5	260.1	0.44
18	" + 45 "	0.0280	4.0	8.2	26.2	26.1	58.4	264.4	0.41
19	Washed	0.0045	3.9	6.4	15.0	34.8	54.3	207.3	0.92
20	" + 15 cc.	0.0136	4.1	6.5	22.9	27.6	61.0	313.9	0.37
21	" + 45 "	0.0328	4.4	6.7	22.4	25.9	62.0	218.4	0.36

Normal acid phosphate added to unaltered table liquor

22	Control	0.0223	3.6	12.2	36.0	35.0	51.4	397.6	1.22
23	+ 15 cc.	0.0297	3.7	12.3	38.6	32.9	52.7	404.0	0.88
24	+ 30 "	0.0381	3.8	12.4	38.0	32.9	52.0	397.6	0.77
25	+ 76 "	0.0500	4.0	13.1	24.1	14.7	68.3	460.6	1.56

Normal lactic acid added

26	Washed	0.0064	3.8	12.7	33.1	48.6	40.1	428.2	2.35
27	+ 45 cc.	0.0273	3.4	12.9	23.5	64.5	25.9	405.8	3.93

Effect of small changes in temperature

28	80° F.	0.0315	3.9	12.8	39.4	28.8	57.6	438.5	0.90
29	85 "	0.0315	3.9	12.8	38.7	29.5	57.2	436.0	1.09
30	90 "	0.0315	3.9	12.8	36.6	24.2	62.3	418.2	1.14
31	95 "	0.0315	3.9	12.8	35.8	22.4	62.8	430.4	0.98

a decrease in density and an increase in phosphate concentration. Experiments 22 to 25 show that separation may be materially improved by adding phosphate to a "normal" mill liquor.

Experiments 26 and 27 illustrate the poor results obtained by adding lactic acid to mill starch that was prewashed to reduce the original content of soluble material.

Experiments 28 to 31 illustrate that slightly better tabling efficiency can be obtained at 95° F. than at room temperatures.

TABLE IV
Influence of Rate of Flow on Efficiency of Table Separation

Experiment No.	Density	Titratable acidity	pH	Soluble material		Flow rate	Gluten fraction		Starch fraction
				Total	Ash		Protein	Starch	Insoluble protein
	° Bé.	N		grains per gal.	grains per gal.	gals. per min.	per cent	per cent	per cent
1	12.6	0.0248	3.7	601	35	2.00	55.8	29.4	0.13
						1.875	58.9	27.3	0.19
						1.75	59.5	26.3	0.29
2	12.1	0.0244	3.8	479	49	2.00	60.6	25.7	0.24
						1.875	63.7	22.0	0.22
						1.75	64.1	22.1	0.23
3	11.0	0.0263	3.8	605	52	2.00	65.5	21.9	0.21
						1.75	63.8	21.0	0.23
4	10.5	0.0228	3.8	488	47	2.00	61.9	21.0	0.10
						1.875	63.9	22.2	0.21
						1.75	64.4	20.6	0.19

Table IV illustrates the wide variations in efficiency of separation obtained with normal mill liquors on a table of plant scale, in mill practice, at varying rates of flow in gallons per minute and at densities between 12.6° and 10.5° Bé.

Again the greater ease of separation at lower densities is apparent by comparing Experiments 1, 2, 3, and 4 at the same rate of flow on the table. But more particularly, the wide variation in efficiency of separation that results with very small changes in the rate of flow should be noted. It is believed that most devices used to regulate the rate of flow on tables of plant scale have not, heretofore, been able to hold variations within the limits of these experiments reported by the writer. This has been due, in part, to the fact that mill liquors of greater density tend to plug regulating valves, owing to the tendency of starch to settle and cake. A satisfactory arrangement suggested by the writer from laboratory experiments reported above consists of a supply tank with continual agitation placed directly above the table with a short and vertical tube leading to a fixed orifice of proper

diameter at the head of the table. It is also to be noted that the faster the rate of flow, in general, as might be expected, the higher the percentage of starch in the gluten fraction, and simultaneously, the lower the per cent of insoluble protein in the tabled starch. Hence, a higher quality starch is accompanied, in tabling at high rate, by a loss of starch in the gluten fraction.

The gluten fraction which is obtained in the separation of starch is recovered. This may be accomplished by allowing the liquors to stand in large tanks from which the gluten gradually sediments. The clear water above in these settlers is withdrawn for reuse. It is referred to as gluten settler or gluten overflow water. The gluten is either dehydrated and mixed with the dehydrated residues from the milling operations, germ residues, coarse grits, and the fine corn bran, together with concentrated steepwater, and sold as a feed for cattle or else all or part of the gluten is further purified and may be used for the preparation of pure corn proteins, zein and zeanin, and their derivatives.

The purity of starch produced may be determined by several practical considerations. They are the relative market price of starch and gluten feed and the demand and current price for the production of pure corn proteins.

The study of the mechanics and physical chemistry involved in starch separation has led to a radical change in milling practice in the last decade. These studies have permitted the use of starch and gluten slurries of much greater density in the tabling operations. The amount of water used in this operation determines the amount of water that must be carried in mill house operations to handle a given weight of corn grain, for in an essentially counter-current wet milling process approximately the same amount of fresh water is introduced in the final washing of the purified starch as leaves the system in the form of steepwater, moisture in the germ, corn bran and coarse grits, and in general evaporation. This is approximately 10 to 12 gals. per bushel of corn of average moisture content. The net result has been that, whereas formerly because of the necessary use of table liquors of 6° Bé. it was found that 30 gals. of water were needed to mill a bushel of corn, with the use of liquors of 12–13° Bé., it is now only necessary to carry 10 to 15 gals. of water in the mill house per bushel of corn.

Obviously, then, in the earlier procedures it was either necessary to discard some 20 gals. of process water for every bushel of corn ground, or else to reuse almost two-thirds of the water in the same milling cycle. Not only was the disposal of such quantities of process waters into sewers prohibited in most localities, but also it was very uneconomical because of a loss in corn solids dissolved in this process water. The tendency was, therefore, to recycle as much water as possible and either to evaporate the remainder as it left the mill house or to crowd it through the steeps, and increase the amount of steepwater that was evaporated. The patents of McCoy (35, 36), McCoy and Sjostrom (37), Moffett (38), Greenfield (39), Jeffries (40–42), and King and Baker (43) illustrate the development of the reuse of process waters in milling and table separations. Recycling, however, tends to increase the amount of dissolved substances to the saturation point. For certain substances which deposit and create a scale on

the evaporating equipment, this procedure created a process water which was very difficult to evaporate. Coupled with this was the fact that there was now more water to evaporate. The result was, in many instances, that the capacity of a plant would be limited by the amount of process water that could be evaporated or otherwise disposed of every 24 hrs. By use of starch liquors of greater density in table separation or in centrifugal separation, however, recycling of water is now reduced to a minimum (44, 45).

The centrifugal separation of starch from gluten, which takes advantage of the difference in sedimentation rate of the heavier starch granules, was proposed in an early patent of De Castro and Muller (46). The method was refined by Kerr (47) and by Schrader (48) to effect a more complete recovery of the starch in pure form. A more elaborate system involving the use of the counter-current flow principle was patented by Peltzer (49, 50) and by Boie (51). The patents of Kelling illustrate the use of centrifugal separation in modern mill practice (52-58). Staley (59) prefers a preliminary table separation before the use of centrifugals.

Among other types of separating equipment and processes is the aeration of the gluten into a foam and floatation. This process may, or may not, involve the use of centrifugals. The development of this process is reported in the references given (60-65). For many years the industry used sedimentation tanks for the starch and drew the gluten and water away from starch after it had settled. Eventually, this method developed into a continuous process in which the basic idea of a cone-shaped separator as proposed by Murdock (66) was used. Later, when tabling became the common practice, these cones were used to concentrate the solids in the gluten effluent from the tables. It is quite possible that the development of continuous gravity separators in the last decade in other fields of industry will again revive interest in further development of this basically sound principle for starch and gluten separation. Another interesting method of continuous separation appears in the literature, which so far as can be ascertained was never practiced. The method involves allowing a suspension of starch and gluten to flow over an inclined, continuous belt which slowly moves in the direction of the starch feed (67). The rate at which the belt moves is regulated by the depth of the starch deposit which forms. The starch is removed from the belt continuously as it passes beyond the point where the starch liquors are introduced. The gluten, in suspension, runs off the lower end of the belt.

Formerly, starch deposits from tables and other sedimentation equipment were, after a light wash to remove a surface layer of gluten, transferred to equipment for further washing by shovels, either by hand or by mechanical equipment. This step involved considerable labor. A very significant advance in practice resulted, therefore, by the introduction of the use of jets of water under high pressure at the head of the tables, to remove the deposited starch by hydraulic means. Behr and Mattheissen (68) apparently conceived the idea, but it was not until the development of the continuous vacuum filter to handle the de-

watering of relatively large amounts of starch liquors that it was found possible to reduce the principle to practice (69).

The tabled starch may be passed over a second series of tables to purify it further or it may be further refined by centrifugation. The use of alkali to refine starch before it is finally freed of water was suggested in an early patent of Hamlin (70). The starch is digested in weak sodium hydroxide at low temperatures and then may be resedimented and neutralized, or neutralized and resedimented. The alkali acts to loosen the last of the small granules embedded in particles of gluten which latter now assume their proper buoyancy. It is possible the alkali may also saponify some of the extraneous fatty constituents of the corn grain, imperfectly separated from the starch in the previous processes.

Finally, the starch is washed with copious supplies of fresh water and filtered. Sherman (71) suggests a washing of ground corn solids directly after steeping. Carried to a logical limit, this procedure might be expected materially to decrease the amount of final starch purification needed.

After being washed, the starch may be dried to make both the powdered and pearl forms of commercial corn starch and the dry modifications of starch such as dextrans, or the filter cake may be passed on to other processes for modifications, some of which are illustrated in the following chapters.

4. Dehydration. Many systems for dewatering and drying starch are in use in the industry. The more common types of filters are the American, Oliver, and String filters. High speed basket centrifuges are also used to remove water. To produce a lump, or so called crystal starch, the starch may be filtered in baskets or porous boxes lined with filter cloths and the water drawn off by suction or filtration. Moffatt (72, 73) makes lump starch by pressing moist filter cake in a cylinder or other device with pressure applied as by a piston. The cylinder of starch is warmed by the use of steam.

The lump varieties of starch are dried in kilns, other forms are dried in kilns, rotary drum driers, specially designed vacuum driers, or on endless belts which pass through a heated tunnel such as the Proctor and Schwartz drier. Normal corn starch moisture is between 10 and 14%. Certain users require a starch of reduced moisture content, which starch may then function as a water absorbent for other products (74). For such reduction in moisture content, powdered starch is usually redried in a Huhn type drier to about 5% of moisture.

The dried starches are then graded according to particle size and packed. Powdered starches are ground over mills such as the Pope mills, and bolted through fine silk sieves.

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CHAPTER III

MANUFACTURE OF MODIFIED CORN STARCHES

After the native starch has been prepared, as outlined in the preceding sections, it is frequently modified or altered in some characteristic to make the starch more suitable for some particular use. These modifications vary in degree from those in which practically no chemical change results in the molecules which make up the starch granule to those in which a significant change can be detected. Indeed, so varied are the treatments in modern manufacture, that it is extremely difficult to classify them and to present a description of the processes in an organized manner. Frequently, moreover, the type of treatment used represents the initial phases of chemical reactions which will be discussed elsewhere. But inasmuch as the alterations to be described are limited mainly to change in physical properties, that is, the resulting product is still essentially starch, they will be grouped under the heading of modified starches.

The treatments to be described apply particularly to corn starch. Many, however, are not limited to this starch, but conditions of operation would quite likely need to be adjusted when the treatment is applied to other types of starches to produce similar effects.

1. Starches Modified in Color. Native corn starch from yellow corn exhibits a light yellowish tinge in reflected light, the color arising principally from carotene, xanthophyll, and related pigments. Most of this color resides in the small amount of corn gluten associated with the starch, as finally separated. It is not considered commercially practical to reduce this residual gluten below a value of 0.25 to 0.30% (estimated as protein, by multiplying the Kjeldahl nitrogen value by 6.25). Quite naturally, therefore, the more efficient the separation, the lighter the color of the final starch product. Furthermore, the color of the starch may be lightened materially by procedures designed to reduce the protein

content. This may consist of simply retabling the starch by suspending it in fresh water at about 12° Bé. and passing it at a relatively fast rate over a second set of starch tables. A more effective procedure, however, is to stir starch from the first tables with water and adjust the pH to about 9.5 with sodium hydroxide. After being stirred for several hours at a treating temperature of about 110° F., it is then retabled, neutralized, and thoroughly washed.

A more perfectly white starch can be obtained by using a chemical bleach. Oxidizing agents are quite effective. But, nevertheless, it is always desirable first to reduce the gluten content to low levels. Otherwise, instead of a brilliant white resulting, with the oxidizing bleach used, grayish tints result.

Corn starch is not bleached as readily in the dry state as might be anticipated. For example, it is the common practice in dry milling to bleach wheat flour with such reagents as nitrosyl chloride, nitrosyl sulfuric acid (1), chlorine dioxide, benzoyl superoxide, nitrogen dioxide (2), diaromatic peroxides such as di-*o*-toluyl peroxide (3), and similar reagents. It is impractical to use these bleaches on dry corn starch, because such large quantities are required to make a substantial improvement in color that the unpleasant odors and taste of the reagents are left with the starch, and in addition the starch may undergo a measurable change in paste properties. Of many dry bleaches examined by the writer only ozone is worthy of mention. This reagent must be used under carefully controlled conditions to secure results approaching those obtainable by wet bleaching methods.

Dry bleaching of starch possesses certain inherent disadvantages. It is the common practice therefore to bleach corn starch in the wet state, *i.e.* before the final drying operation, so that the chemicals in excess of those actually required to produce the desired effect may be neutralized, and the products of the reactions may be washed away to leave a pure starch. Two of the most common wet oxidants used are chlorine or hypochlorite and permanganate. The former has the advantage in cost but requires, for best results, special equipment for handling, which is either non-corrosive or which does not produce products of corrosion that will be taken up by the starch. Bleaching with hypochlorite has the added disadvantage of producing a noticeable change in the paste characteristics of the starch. Also, the action of the reagent is likely to impart a noticeable foreign odor and taste to the starch unless the reaction is very carefully controlled. Furthermore, to produce a starch of extreme whiteness, it is necessary to purify the starch to a very high level before applying the reagent.

On the other hand, the action of potassium permanganate is so highly selective in its oxidizing action on the colored impurities of corn starch that very small amounts of reagent are required. The type of starch-treating tanks, common in most mills, may be used, and no change in starch paste characteristics can be detected after the reaction. A brief description of the process from the patent of Sjostrom (4) follows.

A water mixture of corn starch is made up at about 16° Bé. For each 1000 lbs. of starch treated, 0.8 lb. of potassium permanganate, dissolved in about

4 gals. of water, is added. After being stirred for about 30 min., the mixture turns to a deep tan color, indicating the end of the primary reaction.

About 1.1 lbs. of sulfur dioxide are then added, made by treating 1.7 lbs. of sodium bisulfite dissolved in 3 gals. of water with 0.4 lb. of sulfuric acid at 66° B_e. The deep colored manganese peroxide, a product of the primary reaction of the permanganate on the carotene and xanthophyll, is reduced by the sulfurous acid to manganous sulfate, leaving the mixture finally a pure white color.

The small amount of sulfurous acid in excess is neutralized by adding sodium carbonate until the pH of the liquor is raised to 5.5 to 6.0. The starch is then filtered and the soluble reaction products removed by washing.

The starch is dehydrated, ground, and bolted.

More recently another method of removing the yellow color from corn has been considered. It has been known for some time that these pigments were soluble in non-aqueous solvents such as acetone, alcohol, and so forth. Owing to solvent losses, normally expected in such extraction processes, and the market price of these solvents, this method has been deemed impractical. However, with the steady decline in the market price of ethanol (indications are that the synthetic product would attain a cost of the order of 5 cents per gallon in normal times), the use of solvents and installations for solvent recovery in the corn industries are becoming a common practice.

With such a change in view-point, solvent extraction of starch may very well become a practical process. In a recent patent, Schoch (5) recommends refluxing starch in a water-miscible organic solvent such as alcohol in successive operations until the lipids are removed. In view of reported work by this investigator, it would appear that this procedure is the ultimate in the purification of starch from fatty material, both adsorbed and absorbed, including the carotenoids and alcohol-soluble proteins.

The writer has found, however, that the color of starch may be completely removed simply by washing starch with alcohol-water mixtures at or slightly above room temperature. The alcohol remaining with the starch may be very efficiently removed and recovered by washing the extracted starch with water.

2. Odorless Starches. Whereas whiteness is desired in some industrial applications when starch is used as a size, *e.g.* in white paper manufacture, freedom from odors common to starch is desired in certain instances when starch is an ingredient of a food product. For example, powdered or confectioners' cane sugar shows a marked tendency to cake or lump in storage, particularly in damp climates. Starch, added in very small percentages, is very effective in maintaining the powdery condition of the sugar especially when the starch is predried to low moisture content. But when starch is overdried, for example to about 5% moisture, certain reactions are induced in the constituents of the corn starch granule, so that in time the starch develops an objectionable odor which might be described as similar to rancid oil.

Obviously, these odors might be prevented by extracting the fatty constituents of starch granules with a fat solvent such as hot alcohol, which likewise would

also produce a starch of brilliant whiteness. It has been found by the writer that such odors may be prevented from forming and at the same time a starch of superior whiteness be produced by the judicious use of an oxidizing agent followed by a prolonged drying action (6).

A description of the method of treatment follows.

Carefully washed starch is made up to 16° Bé. with fresh water and, for each 1000 gals., 2 lbs. of potassium permanganate dissolved in about 4 gals. of water are added. The mixture is stirred for about 30 min. at about 100° F. Sulfurous acid is then added in excess to solubilize the manganese peroxide by changing it to manganous sulfate. The products of the reaction and the excess sulfurous acid are then removed by washing. The starch is filtered and carefully dried to about 5% moisture content, ground, and bolted through silk. Normally such a starch has an acidity corresponding to a pH between 4.0 and 4.4. It is now heated with constant agitation, such as in the common type of dextrin cooker, at 180° F. for about 20 hrs. The starch is then cooled, rehumidified to 5% moisture, and packed in suitable containers. It is claimed that such a starch will not develop the characteristic rancid odor given by cereal starches of low moisture content stored for prolonged periods. This is said to be due to the fact that the unstable fatty constituents are oxidized in part by the permanganate and that the reaction is completed by atmospheric oxygen at higher temperatures. A part of these degraded constituents is removed in the washing, a part is volatilized in the heating, and those that remain are apparently stable against further deterioration. Other oxidizing agents may be used to initiate the oxidation of the fatty constituents; *e.g.*, chlorine, ozone, and so forth.

3. Mobile (Dry) Starch. Certain users of dry starch prefer a powdered product, referred to in the trade as a mobile starch. The reasons for one starch being mobile and another not are not clearly understood, and probably a satisfactory definition to cover all phases of mobility could not be given. Evidence of mobility in a starch is shown by one or more of the following characteristics: (a) The starch shows an abnormal tendency to create dust when agitated. (b) It will adhere and spread more evenly than normal starches over certain surfaces on which it is dusted. (c) If loosely packed into a container it will occupy a greater volume per unit of weight. (d) If it is placed on a very fine mesh screen, a larger percentage of starch will pass through the screen for a given amount of agitation of the screen. Some of these characteristics have been used as a basis for devising a test quantitatively to measure relative mobility. These tests are described in another section.

The problem has been critically studied by the writer who has found that among others the following may be listed as the principal variables contributing to lack of mobility: (a) high moisture content, (b) high percentage of ether extractives, (c) high percentage of water extractives which develop tackiness when exposed to a humid atmosphere, (d) electrostatic charge, (e) shape and size of the starch grains or aggregates, (f) prolonged heating in the dry state.

Several attempts have been made to induce mobility in a starch, some of which have appeared in the literature. The earliest method used probably was a thorough washing of the starch by soaking in dilute sodium hydroxide followed by partial neutralization, at least, and then using a copious supply of warm water to complete the washing process. Some improvement will be noted, particularly if the starch is left on the alkaline side, owing possibly to a more complete removal of extraneous fats, proteins, and water-soluble portion of the corn than in a simple washing with water.

Other types of treatment involve a complete removal of some of the interfering substances by washing with a non-aqueous solvent such as hot alcohol or alcohol-water mixtures.

Still another is to pass a purified and highly dried starch over a grounded, metal screen. A more recent variation of this method is to change the sign of the electrostatic charge on the granule (7).

Finally, air separation has been practiced to secure granules of more uniform size and shape.

In most of these methods, however, there is a failure to take into account the fact that of all the variables listed the moisture relationship, in and around the starch granules, is the most important variable. This should not be interpreted to mean that by the addition of an oil to starch, for example, the starch could not be made as dustless or as easily moulded into shape by compression as by the addition of moisture. Starch manufacturers produce the opposite of a mobile starch by just such a procedure for certain uses, particularly for use as a moulding starch. Indentations are made in a layer of the starch and confections such as gum drops are poured from the candy cooking kettle to cool and assume uniform shapes. It does mean, however, that in consideration of a corn starch of the degree of purity now produced by the larger and more progressive mills, moisture relationships are by far the most important variable. This study is admittedly intimately connected with a study of the art of starch drying, as will be evident from considerations given below.

Wet starch, as obtained from the filters in the last washing stage of starch production, may average around 45% moisture. This large percentage is made up of water held in a variety of ways. It is held by capillary action in the empty spaces of the filter cake, it is held as simple surface moisture on the individual granules, possibly some is in a "free" state inside the granule, and, lastly, some is held in a combined state with the molecules of the starch. It is suggested that this latter moisture is held by hydrogen bonding to the otherwise free hydroxyls on the carbohydrate chain, and as such will be referred to as bound water.

Wet filter cake is very immobile. Some 5 or 10% of the moisture, capillary water, can be removed by centrifugation at high speed in a closed chamber. The product assumes a fluffy condition but is still very logy. The next 20% or so of moisture can be removed by careful drying, leaving a starch whose surface moisture is reduced to a minimum and, as first made, the starch is quite mobile but very soon becomes less mobile. If the carefully controlled drying operation

is continued, as over H_2SO_4 in a vacuum at 45°C ., the starch rapidly falls off in moisture content to 7 or 8%, at which level it becomes surprisingly mobile and, if the drying is extended, the moisture gradually falls off to very low levels, 4 or 5%, and a starch of high mobility results. At this stage only a portion of the moisture normally present in air-dried starch remains and this quite possibly is bound water. If the drying is extended to completion by the use of higher temperatures and longer times, irreversible changes are noted in the properties of the starch. For one thing, the absorption of moisture is not nearly as speedily reversed (Samec (8)). For another, it is noted that the starch soon develops a lack of mobility, characteristic of some of the torrefaction dextrins.

The above facts are interpreted to mean that only capillary moisture and surface moisture of the filter cake have a bearing on the mobility of a starch. Internal free moisture is indirectly related in that there is a speedily attained equilibrium between the latter and the surface moisture. Bound water, however, is of no significance as long as it stays bound. The establishment of equilibrium between the most tightly held, bound water and forms of "free" moisture is slow. It might be supposed that the last percentages of bound moisture are held by 6-carbon hydroxyls on the carbohydrate chain, since primary alcohols have greater associative forces than secondary or tertiary alcohols. It might also be calculated by simple arithmetic that possibly as high as 9 to 10% of moisture could be bound in this position, provided each 6-carbon hydroxyl binds 1 molecule of water. More likely, however, the value is probably half of this, owing to the organization of molecules within the granule being such that 1 molecule of water is probably shared by a 6-carbon hydroxyl and some other hydroxyl through another hydrogen bond.

It would seem, then, that all that is required in practice is to dry a pure starch to 5% moisture and mobility would automatically result. Two difficulties are encountered. When the common method of drying the starch in the kiln to about 10%, followed by grinding, bolting, and redrying to 5% in a Huhn type drier is used, the resulting starch is not as mobile as was anticipated from laboratory experiments. Furthermore, the starch soon after manufacture becomes less mobile, which is explained in part by the fact that at normal humidities it tends to increase in moisture content, presumably, at first, on the surface. A more critical examination of the drying process given discloses that at the 10% moisture stage a part of the starch in the carriers in the kiln is severely overdried, having possibly as low as 2% of moisture, whereas another portion may have as high as 18 to 20%. Therefore both of these fractions, that high and that low in moisture, are immobile (although for different reasons). Furthermore, in the redrying process which follows, that portion of the starch which is already too low in moisture content for good mobility is dried to an extent such that it is, in effect, irreversibly modified; a state of equilibrium in the moisture content of this starch at any given humidity can be reestablished only after a long exposure to high humidity or by direct contact with water. In the lot of starch reduced to an average moisture content of 5% it also follows that there must be some

granules with moisture contents higher than 5% and these therefore contain a certain percentage of moisture which is in a flexible equilibrium with surface moisture.

Better mobility at a level of 5% moisture content can be secured by more uniform drying. Starch dried in a rotary drier is noticeably more mobile than that dried in a kiln and a flash-dried starch begins to approach that which can be attained with controlled drying in the laboratory. None of these methods, however, is perfect in eliminating local overheating. In addition, drying to a moisture content of 5% in such equipment involves the constant hazard of fire and explosion during the handling of starch low in moisture content. Moreover, the product is likely to regain sufficient moisture, before it reaches the customer, to lessen its mobility materially.

If the above facts are granted and the above hypotheses assumed to be substantially correct, one solution to the problem would be to increase the capacity of the components of the starch granule to hold more bound water; that is, to activate the starch, so to speak, so that the associative forces for water molecules are increased.

It was found by the writer that treatment of starch with chlorine gas, in controlled amounts, produces the desired result. The method is described in a patent issued to Kerr (9).

Table V illustrates the results obtained. Mobility is measured and expressed as the time in minutes for a given weight of starch to pass the 48 mesh copper wire screen on a shaker sieve attached to an eccentric making 120 R.P.M.

TABLE V
Mobility of Starches

Type of corn starch	H ₂ O	pH	Mobility
	%		min.
Chlorine-treated, 8 hrs. at 125° F.	14.4	5.0	2.5
“ 3 “ “ 95° “	8.9	6.7	4.5
“ 3 “ “ 95° “	12.9	6.1	5.75
“ 3 “ “ 95° “	11.3	5.4	6.0
“ 3 “ “ 95° “	12.3	4.9	7.25
Untreated, flash-dried, air-separated	8.5	5.5	9.0
“ air-separated	10.0	5.5	14.0
Commercial, 5% moisture	5.0		Over 30.0
“ powdered (Sample A)	10.0		“ 30.0
“ “ (“ B)	10.0		“ 30.0

A mobility of 6 min. and under is considered satisfactory for most uses. Two commercial starches (Samples A and B) were prepared by two different manufacturers. The chlorine-treated starches were dried in a rotary and air-separated. The most highly chlorinated starch felt dry to touch, even at 14%

moisture, and the 100 lbs. of starch used in the test fell rapidly through the shakers, filling the surrounding room with dust.

4. Starches of High Viscosity. The chief industrial use of starch depends on the fact that, when starch is mixed with water and heat is applied or certain chemicals added, the starch swells to form a paste. An important feature of this paste is its viscosity or, the inverse function, fluidity. Starches of various viscosities are required for various industrial uses. For most uses starch of normal or reduced viscosity is required. Occasionally, however, uses develop in which it is more advantageous to employ starches of very high viscosity, the higher the better. A few examples of these follow. When starch is used as a thickening agent in cooking, as for instance in thickening soups or canned vegetables, other things being equal, the higher the viscosity of the starch the less starch will be required. Another example is its use when an inexpensive adhesive is essential. Paper bags are sold at a price range in which an inexpensive adhesive, for use in forming the seams, is not only desirable but necessary. Obviously, other things being equal, the higher the viscosity of a starch paste at a given concentration, the more it can be diluted with water to a fixed level for use on the bag-forming machines.

Some starches, when cooked into a paste, exhibit higher viscosities than others at high temperatures, that is around the temperature used in cooking the paste, but either they show no increased viscosity at lower temperatures or else possibly a subnormal viscosity. It does not necessarily follow that, because a starch exhibits an increase in viscosity at one temperature, it will necessarily exhibit the same proportionate increase in viscosity at all other temperatures. Hence it becomes important to describe starch viscosities in terms of temperature and to select the starch for an intended use which exhibits the desired viscosity at the temperature at which it will be used. In practice, therefore, it is customary to speak of hot paste viscosity and cold paste viscosity. The former is often measured by the Scott test at temperatures in the neighborhood of 95° C. A common method of measuring the viscosity of dilute, cold pastes is by means of the Stormer viscosimeter at 25° C. after a stated cooling and aging period. Both of these methods are described in the section on methods which follows.

Several methods exist for increasing the viscosity of corn starch above normal. Some of these, however, possess inherent disadvantages. Perhaps the oldest method is simply to add an alkali or alkaline substance to the starch in sufficient concentration for it to gelatinize with no applications of heat. Very viscous masses result, much heavier in body than obtained by heat gelatinization and cooling to room temperature. Such a paste exhibits a high, cold paste viscosity but rapidly deteriorates into a thin fluid if it is heated at boiling temperatures for any length of time. Obviously the high alkalinity of such a paste is another decided disadvantage for most uses. The alkalinity required to gelatinize starch into a thick viscous mass may be reduced by either partially gelatinizing the starch before addition of the alkaline reagent, or it may be heated in the presence of even less alkali with the production of relatively high viscosities. But in

any event such pastes are quite unstable and lose viscosity at a rapid rate at higher temperatures. They thin out with mechanical agitation required to pump the paste from cooking kettles to other equipment and with the movement of mechanisms used to apply the paste.

It will be appreciated, therefore, that the addition of alkalis to starch does not, according to the Scott test, increase the viscosity of a starch above normal, since the viscosity is determined after a definite cooking and holding period at high temperatures.

One of the more acceptable methods of treating corn starch to increase the hot paste viscosity is with chlorine gas or by the addition of small amounts of hypochlorite. Bryant (10) claims increases in viscosity of the starch result from a limited treatment of corn starch with hypochlorite. Kerr (9) discloses that the hot paste viscosity of a starch may be very materially increased by treating an aqueous suspension of raw corn starch with chlorine gas. The hot paste viscosity of the starch continues to increase with the addition of chlorine up to the limit of saturation of chlorine in the starch slurry. When an absorption tower is used, it is found possible to dissolve 2 lbs. of chlorine in 100 gals. of starch slurry containing 300 to 400 lbs. of starch. After a short holding period, without heat, the excess chlorine is then removed by use of an antichlor, and the starch neutralized and washed free of salts. A starch so treated is claimed to increase in hot paste viscosity, expressed in terms of the Scott test, from a normal of 90 to 100 up to as high as 500.

The cold paste viscosity of the starch described is lower, however, than normal. Less extensive treatment with chlorine, while it falls short of producing the peak hot paste viscosity obtainable by this method, does, none the less, develop an increase in cold paste viscosity of the starch above normal for untreated starch. The use of a more strenuous treatment with chlorine than in the illustration given, by extending the time the starch is in contact with chlorine, results in a gradual falling off from the peak hot paste viscosity to normal and, finally, below normal. Secondary reactions evidently become predominant. One of these is, possibly, hydrolysis, owing to the acidity of the reacting medium; another is the oxidizing effect of the hypochlorous ion.

An additional advantage to be gained in the type of treatment for starch described above is that the product is not only improved in viscosity for such uses as in canning and preserving foods, but also the starch is rendered quite sterile, particularly in respect to resistant organisms, the spores of which are ever present in the atmosphere and which are naturally present on most farm products as they are brought in from the fields for canning. The use of a pure, sterile starch, therefore, lessens the canner's responsibilities in respect to inactivating only those organisms which may be present on or in the other food ingredients, such as the vegetables and condiments, which go to make up the canned food. Kerr (11) has described a method for producing a superior starch of this type in a recently issued patent.

The viscosity of starches may be increased above normal by treatment with other halogens, such as bromine, as reported by Kerr (9).

Another type of treatment, the fundamentals of which are given elsewhere,¹ is pretreatment of starch in manufacture with aldehydes; *e.g.*, formaldehyde, acetaldehyde, glyoxal, etc. A method for treating starch with formaldehyde has been described by Rowland and Bauer (12) who have patented the use of this type of starch in sizing paper during the beater operation. The method is essentially as follows: 1000 lbs. of corn starch are mixed with 1200 lbs. of water in a suitable tank equipped with an agitator. To this mixture are added 10 liters of a 40% formaldehyde solution and 2 liters of HCl (18° Bé.). The mass is held at about 75° F. and agitated for about 24 hrs., or until the desired amount of reaction has taken place. At the end of this time the water is separated from the starch by means of a filter or a centrifuge and the starch is washed several times with clean water. The starch is then dried and is ready for use.

The author and his associates have experimented with a similar process and have noted that the Scott viscosity test of corn starch is raised from a normal of 90 to about 150 or higher by such means. In a limited treatment with formaldehyde the cold paste viscosity of the starch also increases to some extent as in chlorine modifications. As the treatment is extended, with an increase in Scott viscosity values above 150, the cold paste viscosity tends to fall off. But in marked contrast to modification by chlorine, formaldehyde-treated starches show an increased resistance to gelatinization by heat, the pastes are more stable to mechanical agitation and pump pressures, and are much more stable in the presence of alkali than untreated starch. Indeed, formaldehyde-treated starches which exhibit Scott viscosities of about 150 when the pH of the paste is 5.0 actually increase in Scott viscosity as the pH is raised by additions of borax or alkali. Between pH 9 and 11, Scott viscosities of the order of 200 have been observed.

The more extended action of formaldehyde on starch will be discussed elsewhere.

The action of aldehydes on the non-cereal starches, such as tapioca, is unique. By means of a limited treatment, starches may be produced which cook up to cloudy pastes resembling the cereal starches and which set to gels. By inclusion of certain agents in the cooking process, *e.g.* sugar and fruit acid, clear transparent gels result, resembling gelatin desserts. Edible products may be made by the use of acetaldehyde as the modifying agent and elimination of the reagent after modification of the starch by adding bisulfite in excess and by a thorough washing out of the addition products, according to Kerr and Schink (13).

That plasticizers such as sulfonated oils, when added to starch pastes in the preparation of sizes and adhesives, cause a marked increase in the hot paste viscosity, as well as a lowering of the tendency of the paste to gel on cooling, has long been known in practice. That the property is characteristic of all

¹ See Chapter XVII.

dipolar substances, to a greater or smaller extent, has been established in researches conducted by the author and his associates. The higher fatty acids, such as stearic, palmitic, and linoleic, all materially increase the hot paste viscosity of corn starch when added to the starch in small amounts. The use of soaps to increase the viscosity of starches has been studied by Heald (14) and others.

A limited treatment of corn starch with nitrous acid, and neutralizing and washing, have been found by the author measurably to increase the hot paste viscosity.

5. Starches of Reduced Viscosity. While there is a definite market for starches of abnormally high viscosity, most of the modified starches sold are reduced in viscosity below that of native starch. The principal reason is that by so doing more dry substance (starch) may be used in the size, adhesive, or other product, and at the same time the pasted starch product will be fluid enough to be workable. All other factors being the same, the adhesive or binding power, the strength of film, and other desirable properties of this nature increase very materially as the amount of starch increases. The viscosity of a starch paste, however, depends on at least three factors, the apparent viscosity due to swollen granules, the viscosity due to dissolved molecules or dispersed colloidal aggregates, and a viscosity that results from an orientation of these molecules or aggregates. Viscosity may, therefore, be lowered by reducing the tendency of the granules to swell before they rupture, by a reduction in the size of the dissolved molecules, and by a modification of the molecules to reduce their tendency to orient. The second and third methods involve the danger that a decided drop in adhesive strength will follow, owing to a degradation of the starch molecules, for strength in a size or adhesive is, among other things, a function of molecular magnitude. It is obvious, for example, that the lower sugars are weaker as sizing and adhesive agents than the higher dextrans.

A mild treatment with acid is perhaps the most common method to modify starch for this purpose and the products are the so called thin boiling starches of commerce. The method of treating starch with dilute acid at temperatures below the gelatinization point of the starch to produce these thin boiling starches appears to have been introduced by Duryea (15).

The method of manufacture is to suspend starch in dilute sulfuric acid (between 0.1 to 0.2 *N*), warm to about 50 to 55° C., and stir at this temperature until the potential paste viscosity of the suspended starch is reduced to the desired level. The suspension is then neutralized with sodium carbonate, filtered, washed, and dried. A series of products is manufactured showing viscosities from levels only slightly below normal for untreated starch to those low enough to indicate that the product is essentially an acid-modified dextrin. The products are graded on an arbitrary fluidity scale, the higher the fluidity assigned, the lower the indicated viscosity. Untreated starch has a fluidity number of 1, water itself being considered as 100 fluidity for reference.

An alternate procedure used in making the highest fluidity starches is to acidify a suspension of the starch to be treated, filter without neutralizing, and

place the moist filter cake in kilns. Conversion usually requires several days time. This method eliminates the loss of dry substance in the form of water-soluble products which arise in an extended acid conversion. In the method described first, the soluble matter produced in the tub conversion passes out with the filtrate at the filters.

Although the thin boiling starches of higher fluidity, such as 75 and 90, exhibit a reducing value large enough to indicate a hydrolytic scission of starch molecules as the result of the acid treatment, hydrolytic degradation is difficult to demonstrate in starches modified as far as the intermediate fluidities, *e.g.* 40 fluidity. But it should be pointed out that Farrow and his coworkers (16) have suggested that the breaking of a very small percentage of the glucosidic bonds in starch is sufficient to cause a material shortening in the average "chain length" of the molecule.

An examination of a thin boiling starch granule during gelatinization discloses, however, that it swells considerably less than untreated starch. Rather, it disintegrates at an early stage into small fragments (17). It would seem likely, therefore, that the reduction in hot paste viscosity of thin boiling starches is the direct result of the lesser tendency to swell. The reason for the latter may be that there is a limited acid degradation of those constituent molecules on which the tensile strength or elastic limit of the granule mostly depends.

Another consideration favors the view given. It has long been known that while the hot paste viscosity of acid-modified starches is materially reduced, their pastes show a much more decided proportionate increase in viscosity on cooling (18). Indeed so pronounced is the tendency of thin boiling corn starches to set up, or gel, that they are unsurpassed for use in such confections as gum drops. It would appear, therefore, that one effect of the acid is to break off large fragments of some of the branched chain components of the starch, which fragments are sufficiently linear and are of the proper length of chain to orient in paste formation and thus increase the tendency of the starch to gel.

Further support for the explanation proposed is derived from a consideration of the chemistry of potato starch. The conclusion is supported mainly by the fact that potato starch is essentially non-gelling in character and by the claim in a German patent (19) that potato starch may be transformed into a starch which cooks and sets to a short gel by warming a suspension of the starch over an extended period of time with carefully controlled concentrations of acid. Potato starch apparently contains no significant amount of unbranched molecules of the proper chain length for orientation into structures which lead to gel formation, but a very limited scission of some molecules increases the proportion of linear chains of the optimum length, so that when the starch forms a paste, gel formation results.

A second and common method of producing modified starches of low viscosity is treatment of the starch with oxidizing agents. Hypochlorite is quite frequently used for this purpose. This method will be taken up, however, in the section on reactions.

The action of most reagents on starch is attended by a lowering of viscosity. A great many reagents have been used and suggested for use. Even the patent literature is too voluminous to cover completely in this review. Therefore, only a few representative examples will be given.

Salts that can act as oxidants might be expected to accomplish their modification of starch by an oxidizing effect. The patents of Harvey (20), Pierson (21), and others on the use of peroxides and other per-salts are examples of this type. Kerr (22), however, points out an unanticipated effect in the use of calcium peroxide. Whereas most oxidants produce a starch whose cold paste is definitely less congealing when compared to an acid-modified starch of comparable hot paste viscosity, calcium peroxide, on the other hand, produces a starch whose cold paste sets to a gel of greater strength than an acid-modified starch. Obviously such a starch would be more suited for use in making products in which gelling is desirable, as for example in the manufacture of gum drops.

Acid salts, *e.g.* NaHSO_4 , might be expected to modify starch by virtue of the acid activity which develops when the salt is dissolved in water. Salts of weak bases and strong acids, such as ammonium chloride, calcium chloride, and aluminum chloride, which are easily hydrolyzed in water, might be expected to modify the starch for similar reasons.

Frequently, however, there have been reported modifications of the starch that are due to salt effects but are not attributable to the above causes. For example, if very small quantities of an inert electrolyte are present, *e.g.* sodium chloride, certain starches, when they are gelatinized and allowed to stand, lose viscosity at a rate suggestive of a material degradation in the starch molecules. Corn starch is, however, not very susceptible to this effect, but apparently potato starch is (23). So pronounced is this action, that for many years it was argued by Biedermann (24-28), Haehn (29), Iljin (30), and others that the ash of saliva might be the active amylolytic principle. Haehn and Berentzen (31) concluded that a mixture of neutral salt + peptone + amino acids catalyzed the degradation of starch in a manner analogous to the amylases and were, therefore, the active ingredients of amylases.

Such effects, as noted above, in which starch is apparently "liquefied" by contact with small amounts of electrolytes are hardly explainable on the basis of changes in osmotic pressure, within and outside of the starch granule. They are not satisfactorily explainable by a weakening effect on hydrogen bonding in oriented colloidal systems for in this case salts such as NaCl should reduce the viscosity of corn starch pastes more than that of a similarly prepared potato starch paste since secondary bonds appear to be a greater factor in the viscosity of the former than the latter paste but neutral salts have very little effect on the viscosity of corn starch. Further, the results reported would not be explainable by the phenomenon discussed by Zwikker (32), that the alkali salts of amylopectin form less permeable membranes than the salts of the alkaline earths, for when sodium chloride is added to potato starch and thinning results (and if we assume a substitution of the calcium in a naturally occurring calcium salt of potato

amylopectin by the added sodium) the amylopectin membranes should become less permeable and more resistant and the granule and colloidal units should show less tendency to absorb water and rupture, finally.

Samec (8) attributes the high viscosity of potato starch to the ionizable amylopectin-phosphoric acid complex. However, the sodium salt of the amylopectin-phosphoric acid should ionize as well, possibly to an even greater extent than the corresponding calcium salt.

Bugenberg de Jong (33) explains the action by assuming that electrolytes discharge the negatively charged starch particles, thereby lowering the electroviscous effect or part of the total viscosity of a solution. It would seem that this hypothesis justifies further consideration.

Some salts (and organic reagents) are specific in lowering the gelatinization temperature of starch and have been used for this purpose. Samec (34) has reviewed the early literature on this subject and the effect is further discussed in the chapter on hydrogen bonding in starch. Some salts, on the other hand, in high concentration (even the alkaline sodium carbonate) act to repress the gelatinization of starch. Thiocyanates (35) and chloral hydrate (36) have long been known very materially to lower the gelatinization point of starch. Their use for this purpose will be described below. Quite naturally, basic reagents exert a swelling or dissolving action on starch; for example, pyridine, morpholine, the ethanolamines, etc.

Kunze and Evans (37) have advocated the use of dicyandiamide with starch and starch products to reduce viscosity, plasticity, and possible gelling effects in prepared adhesives. This reagent is claimed to be superior to urea and similar reagents, the patent literature on which is very voluminous.

6. Cold Water Paste Products. There is a very definite market for starch products which may be stirred up into pastes or sols with cold water. Quite frequently, in the production of very soluble modifications, the process may be attended by a chemical change in the starch; hence a discussion of the preparation of these products is not logically a part of this chapter.

The most common and perhaps the simplest method of producing a starch product which may be mixed into a paste with cold water is to pregelatinize the starch, or partially gelatinize it, with water and heat and then dry it. Corn starch products of this nature are sold under the trade name Amijel. Similar products prepared from tapioca and potato starch are Tapinol, Tufgel, etc. Their principal use is in sizing paper pulp at the beaters. The starch is introduced into the pulp beaters and the action of the beaters redisperses the starch to effect a sizing of the pulp fibers.

There are several variations in procedures for making such products. The simplest method is to pass starch filter cake, having a moisture content of from 40 to 50% following the milling operation, between heated rolls which are balanced in speed and temperature according to the moisture content of the starch and its ability to gelatinize. The latter characteristics will be determined by the variety of starch used, whether corn or potato, for example, by whether additions

have been made to the starch to alter its gelatinization, or by whether the starch may have been premodified by a slight chemical treatment.

A thin, gelatinized sheet is produced which is removed from the roll by knives. The product is then adjusted to the proper moisture content, ground, and bolted.

A variation of the process, by which a more thorough gelatinization of the product is secured, is to feed a water suspension of the starch, *e.g.* about 4 to 5 lbs. of starch per gallon, to the heated rolls. Another variation is thoroughly to gelatinize the starch product with heat and sufficient water and then spray-dry the product or dry by some means suitable for the economical dehydration of viscous masses. Obviously, other things being equal, the extent of gelatinization during the process will depend on the water available at the point of heating. On the other hand, the more water there is to be removed by evaporation and drying, the more costly the process. To secure a better degree of gelatinization a mild alkali may be incorporated with the starch in small amounts; *i.e.*, sodium carbonate, phosphate, or borax. The latter also serves as a wetting agent when the product is redispersed in water. Other wetting agents may be incorporated when a slight alkalinity is not desirable. Alkalinity serves another purpose, however, in that it tends to reduce the retrogradation effects which follow when starch is gelatinized and dried.

Some retrogradation is always shown by products made from unmodified starch in that when they are mixed with water they do not redisperse into pastes comparable with those from freshly cooked raw starch. For some uses a minor amount of retrogradation is not objectionable, indeed, even desirable, as will be evident from a study of uses. When retrogradation is not desirable, the starch may be first modified to reduce retrogradation effects.

In one commercial process the starch is first modified by a slight acid treatment in aqueous suspension. The mass is then fed to heated rolls. Some hydrolysis in the process is evident from an analysis of the product which shows about 5% reducing sugars estimated as dextrose. One such commercial product, sold under the trade name Amidex, is used as an adhesive.

To improve the characteristics of starch products that swell in cold water, the addition of lecithin has been advocated by Sichel (38), while Moller (39) suggests the addition of lipids.

Kesler (40) proposes a novel process of stirring a starch suspension while heating and including an agent such as hexalin which presumably limits the swelling of the granules. Heating is continued until the granules lose their characteristic crosses in polarized light. The water content of the slurry is such that at this point the mass is plastic. Heating is discontinued and the mass is stirred until dry enough to complete the dehydration by transferring to normal starch-drying equipment. According to another patent a similar process (41) involves the use of soaps, sulfonated tallows, and oils in amounts varying between 1 and 30%.

Bauer (42) describes a method of treating mixtures of moist starch and urea with steam and then drying and grinding the mass.

More elementary variations in the process for producing a starch that swells in cold water include the following. Stutzke (43) would spray a starch solution into a superheated chamber, whereas Maier (44) would spray starch into superheated steam. Hoppler and Haake (45) claim a starch swelling in cold water may be made by passing starch of normal moisture content between plates at a pressure of 2500 kg. per sq. cm. and a temperature of 150° C. Gill (46) suggests a process for extruding starch at 13 to 17% moisture, under pressure through orifices 0.012 to 0.026 in. in diameter.

Several radically different methods have been proposed. Kantorowicz (47) has described the treatment of starch with alkali in alcoholic suspension. One-hundred parts of pulverized starch are mixed with sufficient alcohol to form a milky fluid. Forty parts of a solution of sodium hydroxide (30° Bé.) are then added and the mixture is allowed to stand for an hour. The alkali is neutralized with acetic acid and the starch precipitate filtered, dried, and ground. It is claimed that the product will give a fluid paste upon the addition of cold water. In another patent, Kantorowicz (48) describes the use of acetone or mixtures of ether and alcohol in place of the alcohol. Dritter (49) describes treating starch suspended in a halogenated organic solvent, adding sodium hydroxide, removing the solvent, and then neutralizing. Supf (50) has patented the gelatinizing effect of thiocyanate. He would treat starch in an alcoholic solution of thiocyanate and then wash the starch thoroughly with alcohol. French and Gill (51) described a process for heating starch in glycerol. If carried too far, as for example a prolonged heating at 200° C., then, as shown by the older work of Pictet (52), the product passes beyond a soluble starch stage, and depolymerization reactions produce substances which Pictet called hexosans.

Finally, as an illustration of how extensively early investigators explored the field of making starch products which disperse in cold water, attention is directed to several early patents of Anderson (53-55). The essentials of the process are to treat starch (air-dried) in a closed vessel for 10 to 45 min. at temperatures varying between 125° and 300° C. The pressure is suddenly released, which causes the granules to swell and burst. This action is probably due to the release of water, within certain parts of the granules, which has been vaporized by the heat and confined by the external pressure. It is claimed that the porous, dry mass dissolves or disperses in cold water and forms an excellent paste or size for laundry or similar purposes. This process is similar to one that has been used for many years to produce breakfast foods from cereals, such as rice and wheat, which were described as "shot from guns." It is interesting to note that the production of an exploded starch is contemplated in a brief review given in a recent note in *Science and Appliance* (56).

7. Modification of Starch with Gluten. The character of a starch product or paste may be modified by the presence of various proportions of gluten or other protein. Alexander (57) has described the early use of flours, such as

wheat flour, as adhesives. A considerable quantity of wheat flour of low grade is consumed in making an inexpensive adhesive for wall paper and the sealing of the seams of paper bags.

Another modification is a roll-dried product that disperses in cold water made by passing a mixture of corn starch and corn gluten over heated rolls. In one procedure use is made of the finely prepared intimate mixture of the two, obtained from the wet mill houses where corn starch is manufactured, just prior to the stage at which these liquors are passed over tables or through centrifuges to separate the starch. One type of product is sold under the trade name of Mogul (58-60). It is used for such varied purposes as an adhesive, as a core binder (mixed with sand in forming foundry moulds), and as a coagulant for certain mine wash waters.

Another manufacturer varies the process (61) by heating the suspension of starch and gluten up to about 152° F., with agitation, then filtering and drying. By the addition to such suspensions of further quantities of gluten products are obtained that have a protein content ranging from 5 to 50%. The product is claimed to be suitable for use in processing canned foods, since when it is used as a thickening agent the water-carrying ability of the thickener is not so extensively reduced in the cooking operation as when starch alone is used.

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declined. Whether this is a temporary condition or whether tapioca will be permanently displaced by domestic substitutes remains for the future to decide. It will depend primarily on American ingenuity in developing satisfactory starches from the waxy variety of grains (such as sorghum and maize) and from roots and tubers (such as sweet potatoes and white potatoes) at a cost low enough that our legislative bodies will be inclined to regulate the imports of tapioca starch. It may also depend on the further development of the manufacture of tapioca starch in the Americas during the present interval.

2. The Raw Material. Tapioca starch is produced from the tuberous roots of the *Manihot* plant, which is cultivated principally in the East Indies. Although the climate and soil of Java seem to be admirably suited to its growth, which conditions account for the large proportion of tapioca starch produced by the Javanese, the plant grows well in many tropical and semitropical countries. It was originally a native of Central America. Hence, a variety of names have been applied to the plant, depending on the locality in which it is grown to produce the starch, cassava, manioc, and yuca.

There are two general varieties of the plant, *Manihot utilissima* and *Manihot palmata*. The former contains a higher percentage of starch and, hence, is cultivated by plantations which supply the more modern and progressive mills. The manufacture of starch from this variety is complicated, however, by the fact that these tubers contain 0.01 to 0.04% of a glucoside called phaseolunatin which yields hydrocyanic acid during the milling operations, by the action of an enzyme which is present in the juices of the tuber. Unless the processes are designed to prevent it, the result is a blue-colored starch, produced as the result of a reaction between iron and hydrocyanic acid to form a ferrocyanide. Although both varieties of *Manihot* roots contain the hydrocyanic acid glucoside, the latter is concentrated principally in the outer layers of the *Manihot palmata* root and is, therefore, more readily removed by peeling the skins from the roots. The so called sweet variety is consequently used as native food and in some of the more elementary milling processes. Some botanists believe the sweet variety developed during the cultivation of the bitter. Furthermore, it is to be noted that transplantation of a given variety into a totally different climate and different soil may cause a marked change in the nature of the plant, especially in respect to the amount and distribution of the hydrocyanic acid glucoside (2).

Manihot roots cannot be stored unless they are thoroughly desiccated. The enzymes present in the roots become very active as soon as the roots are dug. In certain sections, as for example, the East Indies, where labor is cheap and abundant, the roots may be cleaned, ground, and spread out in the sun to dry and may then be used for starch manufacture at some later date. Ordinarily, however, cultivation of the roots, harvesting the crop, and milling the starch must be coordinated and this accounts for plantation ownership and operation by some of the larger millers of tapioca starch. A substitute system is for the millers to contract with independent farmers for the production and delivery of stated quantities of roots. This alternative has certain advantages that will

be seen from the following considerations. The *Cassava* plant is very sensitive, among other things, to moisture conditions. It can endure neither standing water nor periods of drought. Especially during the dry periods, marked changes in the starch content of the tuber will be noted. Thus, should unfavorable conditions prevail during the season in the particular location of the plantations, the raw supply of the industry would be jeopardized, whereas if the supply is drawn from small farms, in scattered localities, assurance of a supply of roots is more certain.

In the case of small farms, however, considerable variation in the raw product may be found. Not only will the size of tubers and starch content vary, but, what is equally important, the quality of the starch exhibits variations as well. In many sections of the tropics, *Cassava* occupies much the same position as potatoes do in some parts of the temperate zones in being the principal carbohydrate of the daily diet (1). The roots may either be boiled, fried, or dried, and made into a flour for baking. Among the native peoples, there are as many individual preferences for a particular variety of *Cassava* as there are among those who rely on potatoes for daily sustenance. The native farmer has special names for most of these minor varieties but the names give no assurance of distinguishing them for starch production purposes. The names which are used to differentiate these varieties vary with locality and are usually based on some superficial characteristic of the tuber when prepared for eating.

Considerable variation in tapioca starch results also from the fact that no small share of East Indian tapioca starch originates from small mills (3) where the starch is made by crude equipment from different varieties of *Manihot* and in uncertain states of purity and virginity. This product is transported to local markets and to larger mills for further refining.

Although analysis of the tubers will show considerable variation, the figures given in Table VI may be taken as average for the common varieties at harvest time. A comparison is made with the average content of common varieties of potatoes (4).

TABLE VI
Composition of Manihot and Potatoes

	<i>Manihot</i> roots per cent	Potatoes per cent
Moisture	70.25	75.80
Starch	21.45	19.90
Sugars	5.13	0.40
Proteins	1.12	2.08
Fats	0.41	0.20
Fiber	1.11	1.10
Ash	0.54	0.92

In comparison, *Manihot utilissima*, the bitter variety which is cultivated for starch manufacture, usually averages around 30% starch at harvest time (5).

The starch granules are contained in the cells of the pith of the tuber, as they are in potatoes. However, in contrast to potatoes, the *Manihot* cells are smaller, the cell walls are thicker, and the starch granules are smaller. In both cases it is necessary to rupture the cells in order to free the starch. Any cells escaping rupture will carry their starch content to the fibrous residue, and the yield of starch produced will be decreased, proportionately. It is more difficult to fracture the cells of the *Manihot* root and hence extract all of the starch than to rupture the cells of the potato. Therefore, when highest efficiency was required, the mechanical equipment used in the older art of potato manufacture needed some elaboration when applied to the manufacture of tapioca starch on an industrial scale.

Another difference is to be noted. Although it is desirable to clean potatoes thoroughly before milling, so that separation of a white starch is facilitated, it is essential that all fine dirt be completely removed from the tapioca roots before they are processed. It is almost impossible to make a starch of prime color otherwise. This is partly due to the fact that, as the individual granules of tapioca starch separate, quite frequently a concave region develops on one face of the granule, in which depression fine particles of dirt may lodge and not be removed by simple washing. Peeling of the roots need not, however, be complete before milling.

3. Production Methods. There are two general methods for rupturing the cell walls to release the starch, biochemical action and mechanical means. In the former method, the roots are allowed to ferment or decay up to a certain stage. They are then pounded into a pulp, and the starch is washed from the pulp with water. The latter method includes breaking the cell walls by scraping, rasping, or crushing the roots.

The practice of allowing the roots partially to ferment or decay was employed by the Chinese of the Malay States during the nineteenth century and by small native millers in other sections (1). A rather complete separation of starch from fiber pulp can be accomplished by such a pretreatment, but the over-all yield of starch, nevertheless, is not materially increased, and the quality of the starch is inferior. Hence, practically all tapioca starch now produced is made by mechanical disintegration of the tuber cells.

In the most elementary case, such as the small native manufacturer who produces enough for local demands or for transport to larger mills for further refining, the miller has little else than a grater. This is usually constructed from a piece of tin, in which a number of holes have been made, the upstanding edges of these holes forming the grater. Many of the small graters of the native Javanese are constructed from a small board in which copper or iron wires are fastened to make, in effect, a metal brush. The next improvement in native production methods was the tread-grater. This consists of a hollow cylinder attached by wooden spokes to a center axle. The cover of the cylinder is made from strips of tin which are provided with teeth. A wheel attached to the cylinder is set in motion by a pedal. As the native pedals the grater into motion,

he presses the *Cassava* root against the grater, while a stream of water out of a reservoir runs the pulp into a basin underneath the wheel. The grater is provided with a wooden cover to prevent loss of materials by splashing.

The starch is allowed to settle in a basin, the pulp decanted, and the settling process repeated several times with fresh water. Finally, the settled starch is allowed to stand to form a compact cake. It is then placed in bamboo baskets, in the sun, in order to dry.

The grating machine or pulper used in Javanese tapioca mills may consist of a revolving box or drum, running at high speed and covered with perforated sheet iron. As a rule a so called re crusher will be found under the grater box to complete the disintegration of the cells and insure a maximum yield of starch. Some of the larger mills employ saw-toothed rasps similar in some respects to those used in the manufacture of potato starch; *e.g.* the Jahn Rasp. It is impossible, however, even with efficient grating devices to remove all the starch in a single operation. In order to recover some 10 or 15% of the total starch which remains with the fiber, the pulp is passed through a so called pulp mill. This consists of a special fine mesh sieve through which the pulp is rubbed and which requires a relatively large expenditure in motive power.

After leaving the grating machines, the pulp is washed with fresh water over sieves of sufficient fineness that the coarse pulp and unopened cells are removed, but not the finest pulp and starch. As a rule, two or three sieves of different meshes are used to facilitate the process.

Of the four types generally used in Java, namely rotating cylinder sieves, shakers or vibrating sieves, brush washer sieves, and fixed or stationary sieves, the first is probably the most popular. The reasons are that (a) various gummy materials which tend to agglutinate with the fiber continually plug the meshes of all sieves. But the rotating cylindrical sieves tend to free the meshes as the cylinder rotates. Once each cycle the sieving surface is inverted. The shakers, or fixed sieves, remain in a horizontal or slightly inclined position. They must therefore be washed frequently by stopping operations and hosing with wash water. (b) The shaker sieves, especially, need more repairing and are more costly to operate. (c) The fixed sieves are used in factories of low capacity, and where space is limited. They are, however, less efficient than either of the other types.

A few of the more progressive mills use a fourth type of sieve known as the flat brush washer. In this the pulp is discharged into the center of the sieve, where brushes gradually push it in a spiral line to the circumference. The pulp in the meantime is continually sprinkled with water from numerous sprays, so that the action of the brushes not only tends to facilitate washing the starch from the pulp but also aids in keeping the sieve holes open. The residual pulp, which comes to the edge of the sieve, is swept into a duct, by the outer brushes. An intermediate type is the cylindrical sieve with brushes.

Complete removal of the coarser fiber is essential to the production of first quality starch. Larger particles are not removed in subsequent starch settling

operations. They not only contaminate the flour by their presence but also interfere with the more complete separation of fine pulp from starch when the latter is allowed to separate by gravity.

In order to separate the starch from the strained slurry, the latter is carried off to settling tanks and allowed to stand or is slowly run down inclined troughs, called tables. The first consist of cemented basins with a flexible arrangement for drawing off the supernatant liquor containing the light pulp. The latter is run into a second settling tank to recover an additional amount of starch which settles out.

The settled starch is then resuspended in fresh water and allowed to resettle. Resuspension is usually accomplished by an agitator consisting of two wooden beams, the radius of the tank in length, which are capable of floating on top of water which is run onto the starch. They are united in a bearing which moves up and down, depending on the water level, along a central axle. Motivation is accomplished by a drag chain attached to an overhead wheel.

Settling of purified starch depends on electrolyte concentration, other factors being equal, and according to unpublished observations of the writer, the anion is more effective the higher its valence. Phosphate is quite effective. However, small additions of sulfuric acid are sometimes used in the manufacture of tapioca.

Considerable care should be exercised in controlling the final pH of the finished dry starch, which should be about pH 5 to 6. When hard or alkaline water has been used for the final purification, traces of sulfuric acid may be an added advantage in this connection also. The purest starch is made by using liberal amounts of naturally soft or softened water. Extremely hard water used in processing (high in lime) has been known to leave calcium oxalate in the finished product.

Tabling and the influence of soluble electrolyte thereon have been discussed under corn starch manufacture.

Some of the more modern factories use centrifugal separators, particularly for the second or final separation. The impurities in colloidal suspension are more effectively eliminated, and the separators have the added advantage of producing a drier starch cake for the final drying operation. The moisture content in such starch cake is frequently reported to be under 40%. This content is not as low as can be obtained with corn starch when centrifugation is used in the final step but it is 10% lower than can be obtained by simple settling of starch and 5% or so lower than can be obtained by filtration. This reduction in moisture content is obviously an added advantage in speeding up the drying operation.

In the smaller factories in Java, drying the starch in the sun has been used extensively. This procedure has two advantages: the low cost and the bleaching effect exerted by the solar rays on the starch, which effect is not obtained in drying in ovens or in kilns.

However, in the more modern factories, mechanical drying is now used exclusively. The systems used vary considerably, even more than in the manu-

facture of corn starch in America. They vary from crude open hearths in which the starch is spread out on a galvanized iron plate over a low wood fire, and continually turned over by hand, to a more or less automatic and continuous drier which is strikingly similar in some respects to the Proctor-Schwartz drier recently adopted in the United States for the manufacture of corn starch. The starch is fed automatically onto a continuous cloth belt which travels at a determined rate of speed through a drying tunnel. Instead of forced hot air drying, however, the heat is supplied by steam-heated elements underneath the conveyer belt. This method possesses three major advantages, quick drying, the small space required, and practically an elimination of biochemical activity during the drying operation.

When mechanical drying is used, chemical bleaching is frequently resorted to in the final stages of production before drying. Because of the nature of the colored impurities in tapioca starch, which are mainly extremely fine pulp particles, in contrast to the carotenoid material in cereal starch, a reducing rather than an oxidizing bleach is more effective. Bisulfite is frequently used for this purpose.

After the drying operation, the starch is sent to mills which break up the granule aggregates, and it is then bolted over sieves and packed for the market. Several grades are usually produced. There is a prime starch grade and a secondary grade, the basis of which latter is the product obtained from the second settling or retabling of the *Cassava* pulp.

The pulp residues are sometimes combined and sold as a carbohydrate feed for cattle.

4. American Production. Although Java has produced most of the tapioca starch of commerce, some starch has been manufactured in Europe (6) from imported, dried roots, and increasing quantities are being produced in the western hemisphere. In the latter instances greater over-all plant efficiency is maintained, necessitated no doubt by higher operating costs. It should be noted, for example, that the manufacture of tapioca starch reached a surprisingly high state of efficiency in Florida in the first decade of the twentieth century (7). These mills were the first to introduce and improve on the efficient rasping devices used in the manufacture of potato starch. Even so, apparently, Florida was not able to compete with Java, owing to the wide difference in labor costs. During the last decade, however, the manufacture of tapioca starch has developed again in the Americas, particularly in Brazil and in the Dominican Republic (8). Examples of American methods of manufacturing will be given to illustrate means of obtaining a high degree of over-all operating efficiency.

A Brazilian plant near Cacapava receives roots in sacks by truck or rail from outlying farms. The supply is both from plantations owned and operated by the starch company and from independent farmers. The roots are dumped by hand into a receiver which feeds rotary washers capable of handling about 30 tons per 24 hrs. The latter are about 3 ft. in diameter and 12 ft. long and are of perforated steel with horizontal openings about $1\frac{1}{2} \times \frac{1}{2}$ in. Inside are helical

shovels, about 5 in. high, that move the roots forward and toss them. Two 2 in. water spray pipes run inside the length of the washer. Naturally soft, surface water is used throughout the process. The roots leaving the washer are clean of all dirt particles and about 80 to 90% peeled.

At the discharge of the washer is a Jahn rasp which in turn empties into a tank. The latter feeds a coarse sieve of perforated copper. The sieve is U-shaped, about 30 in. in diameter and 12 ft. long, and is stationary. A rotating shaft and paddle arms inside move the coarse pulp forward under a spray of water and finally discharge it into a second rasping machine. The washing process is then repeated on the reground pulp.

The starch liquor now goes over a series of copper shakers on each of which there are pipes that spray water to wash the starch from the finer pulp. The final starch liquor at about 3-4° Bé. is pumped to starch tables about 10 in. wide and 80 ft. long, and the last of the fine pulp runs off, leaving the sedimented starch in a rather high state of purity. The starch is now made up with fresh water and sent by pump to cloth-lined basket centrifuges in order to remove the last soluble impurities as well as the fine, colloiddally dispersed material. The baskets are about 3 ft. in diameter by 2 ft. high, with a capacity of about 130 lbs. of starch. The filling and spinning cycle is about 15 min. on each basket.

The centrifuged starch is fed to vacuum driers about 4 ft. in diameter and 16 ft. long which operate at about 17 in. vacuum and 55° C. and require a 90 min. cycle to dry about 1500 lbs. of starch to about 14% moisture.

The driers discharge into an elevator to a Pope mill and bolting reel, from which the powdered starch is packed in bags.

Not only is prime starch made by such a process, the wet end of which is operated in the cold, but high recovery of starch is obtained as follows: The effluent from the tables is used to form a slurry with the washed coarse and fine pulp. This is autoclaved with a small amount of sulfuric acid, then blown into wooden tanks, and neutralized with sodium hydroxide or carbonate. It is fermented with yeast in the usual fashion. After a crude recovery, the alcohol may be refined in fractionating stills or fermented further to acetic acid, depending on the relative market price for the two commodities. The crude acetic acid is concentrated and crystallized as the sodium acetate, from which acetic acid of high strength is obtained on treatment and distillation with sulfuric acid, in the usual fashion.

The yield of starch averages about 20% of the weight of the roots ground. Practically the entire remainder of available starch can be accounted for in the production of the secondary products mentioned above.

One of the largest and most modern, tapioca starch factories in the western hemisphere is not far from continental United States. It is located at Quinigua, in the Dominican Republic. It was built and operated by a subsidiary of the Corn Products Refining Co. which owns plantations for growing *Manihot*. It has been estimated that this plant has exported about 5 million pounds of tapioca annually to the United States (1).

Both systems of maintaining a supply of roots have been used, the plantation method and the colono system. Fig. 42¹ shows a section of the 8000 acre

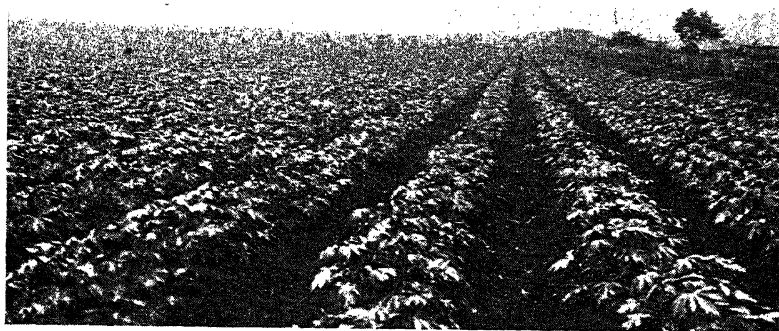


FIG. 42. Plantation of young tapioca plants

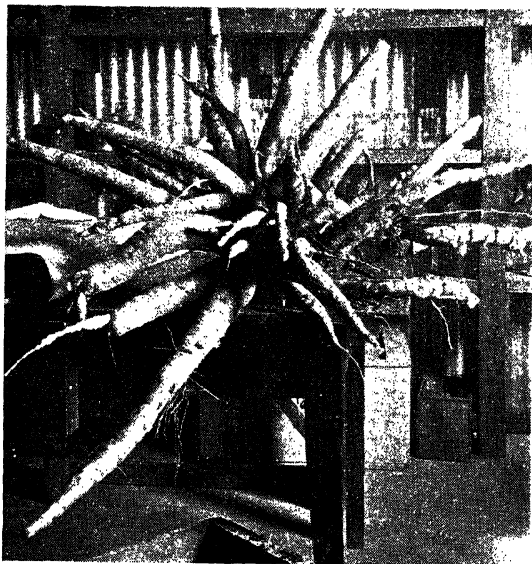


FIG. 43. Tapioca roots, showing whorl formation

photographs shown are by courtesy of H. T. Middleton and C. R. Ridgway and the "International News" of the Corn Products Refining Co.

plantation owned by the company which operates the mill, with young plants set out in orderly rows. With cultivation, these plants grow to a height of 5 to 8 ft. (although 12 ft. is not uncommon) in 14 to 16 mos. Cultivated plants grow large whorls of roots as shown in Fig. 43. These will average 16 to 18 in. in



FIG. 44. Tapioca starch factory at Quinigua, Dominican Republic



FIG. 45. Special tapioca root washers

length and about 5 in. maximum diameter. The colono system consists of contracting with small farmers to grow roots on their own farms.

Fig. 44 is a picture of the Quinigua plant. Corrugated sheet iron, covered with aluminum paint is used to deflect the sun's rays and withstand tropical weather. The windows are without glass but are provided with shutters which

may be closed in the event of a hurricane. An excellent yield of tapioca starch of the highest quality is produced by machinery, such as the specially designed root washer shown in Fig. 45, special Jahn, saw tooth rasps, and large high speed centrifuges for final purification of the starch after tabling.

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CHAPTER V

MANUFACTURE OF WHEAT, POTATO, AND OTHER
INDUSTRIAL STARCHES

The essentials of the manufacture of other starches of industrial importance in the United States, such as wheat and potato, have been described in detail elsewhere (1-5). A brief review of these will be given, illustrating the development of the art in the United States, to supply a background for our present research on the process and the improvements that may be reported from time to time. The manufacture of other starches which are or which promise to be of industrial importance, such as sweet potato, sago, and arrowroot, has been less extensively discussed. Of the waxy starches which may be developed to replace the now less available tuber starches, the newer hybrids of waxy maize appear to be of such character that they may be milled by procedures and equipment comparable to those used in the milling of ordinary corn grains (6, 7).

1. Wheat Starch. Wheat starch is probably the most difficult of all starches to prepare in high yield with a reasonable degree of purity, which difficulty accounts, in part, for its relatively high market price in normal times. Complications arise in the manufacture of wheat starch owing to the tendency of the wheat gluten to become a sticky, dough-like material in the presence of small amounts of water and a smeary or slimy mass in the presence of larger amounts of water. The various processes that have been developed to separate wheat starch differ principally in the manner of conditioning the non-starchy components of the grain to facilitate their separation, particularly, the gluten.

Whole grains are used in the older processes and dry milled flour in the more recently used methods for starch separation.

The whole grains vary in analysis, depending on the variety of wheat and its moisture content. However, at an average moisture content of 14%, the following percentages may be taken as average for the other constituents: protein 12.5, fiber 2.5, ash 1.75, fat 1.65, sugar and gums 3.6, and starch 64.0. Dry milled flour usually contains less than 1% fiber, about 1% fat, 0.5% ash (unless salts have been added to the flour), and approximately 70% starch.

Early Methods. If whole grains are soaked in water and then pounded or otherwise crushed and worked up into a dough, it will be found possible to separate a considerable portion of the starch by transferring the plastic mass to a muslin bag and kneading the dough in a vessel containing some water. A good share of the gluten, together with the hulls, remains in the bag, whereas a part of the starch and some small particles of gluten pass out into the water. The suspension may then be stirred up and passed over a cloth filter to remove more of the gluten, whereupon the filtered starch may be recovered by allowing it to settle from the suspension and by decanting off the water. This or some modification of this method probably constitutes one of the oldest processes for preparing starch. Certainly the extraction of starch from wheat was known to the ancient Greeks and their name for starch, *αμυλον* (*amylon*), signifies that starch, in contrast to wheat flour, was produced without milling. Marcus Cato, in his "*Scriptores Rei Rusticae*," a treatise on Roman agriculture, describes a method of manufacturing wheat starch similar in essentials to the procedure outlined above.

Alsatian Method. It will be found, if the grains of wheat are soaked in warm water, in the experiment described, that the crushing process is facilitated and that a higher yield of better quality starch is obtained. This innovation is the basis of the so called Alsatian process. The grains are steeped in water at about 30–35° C. for 24 to 48 hrs. The water is changed frequently to wash away soluble material and to prevent the development of high acidity. The grain is crushed after steeping, either between mill stones or channelled rollers. Crushing should not be so strenuous as to cause an unnecessary disintegration of the hulls. The mash is transferred to an extractor, which may consist of a finely perforated trough with rotating arms on a horizontal shaft which is used to knead the doughy mass. During this stirring process, a sprinkler pipe above the trough supplies a continuous spray of water which washes out the starch. The starch falls into a collecting trough under the perforated one.

The slurry, containing starch and some small particles of gluten, is passed over a series of shaker sieves in order to remove the gluten. After this operation, the starch is further purified by passing the slurry over starch tables or to centrifugal separators. The Alsatian process produces starch in yields of around 45%. An additional 10 to 15% of second grade starch may be obtained by reprocessing the residues from the first extraction. The final residues, consisting of hulls, wheat germs, gluten, and some residual starch are said to be suitable for use in animal feeds only. It is quite difficult to separate the gluten from the

residual mass. Occasionally this is done but yields are low, seldom averaging over 5%.

A variation of the above process combines crushing with extraction. Several pairs of roller crushers, one above the other, grind the steeped grain, while water sprinklers above the rollers wash the starch away from the hulls and gluten which collect on sieves. The collected residues are usually milled a second time, so that more starch can be separated.

Second or low grade starch obtained in this process may be further purified to remove residual gluten. Agents are added which tend to swell or dissolve the gluten. For this purpose, either weak acids or alkalis may be used; *e.g.*, acetic acid or ammonia. Or the starch may be transferred to fermentation vats where, after a partial degradation or dissolution of the gluten by fermentation, the latter is more easily separated from the starch by washing and sedimentation.

Halle Process. In the Halle, or fermentation process, a fermentation step is employed either during steeping or directly after the steeped grains are crushed or in both steps of the process. In any event, the fermentation is prolonged, requiring about a week at 25° C. The action is induced by seeding a new mash with a portion of the sour liquors from a preceding batch. Alcoholic fermentation occurs during the early stages, then acid-producing bacteria create mixtures of acetic, lactic, and butyric acids. Finally a putrefaction sets in, producing rather offensive odors from protein degradation products. The odor is supposedly an index that the desired action on the gluten has been obtained and the fermentation is stopped lest the gluten be further transformed into a ropy or viscous mass.

After the fermentation, the sour liquors are removed, and the starch is extracted by washing. The latter is accomplished with greater ease than in processes in which fermentation is restricted. Yields of 60% of starch low in protein may be obtained by transferring the mash to a closed washing drum which rotates slowly on a horizontal axis. The outer surface of the drum is usually constructed of finely perforated metal or cloth sieves. As the drum rotates, spray water is introduced through pipes, passing through the bearings. The starch is washed out and is collected in a trough underneath the drum. It will be seen that this method is time-consuming, and the process does not permit recovery of the gluten from the wheat in a commercial form. The mills using this process often reek with ill-smelling odors, and of course losses in yield are very considerable. The sour residues are not preferred for cattle feeding. For these reasons, even though the Halle process was used almost exclusively for many years, it has now been superseded almost completely by other methods. Pure wheat gluten and products derived from wheat gluten now command such a price as to make complete recovery of the gluten very attractive. Indeed, in some instances the production of gluten has become the major objective, wheat starch assuming the position of a by-product.

Martin Process. One of the older methods used, which leads to the production of a gluten of high purity as well as a starch of high quality is the so called

Martin process, invented in Paris in 1835. Dry milled wheat flour is worked into a dough with about 40% of its weight of water. Dough mixers, such as are used in large bakeries, are suitable. After standing for an hour or more to hydrate the gluten, the dough is cut into large lumps. These are kneaded and rolled for an extended period of time on a grooved bed. As the dough is worked, overhead jets of water slowly wash out the starch through sieves flanking the bed, to troughs which lead to starch tables or centrifuges.

Yields of gluten amount to 10 to 15%. Yields of starch of prime quality average about 55%. In addition a yield of about 20% of a starch high in protein is obtained, which may either be sold as such for certain uses or may be additionally treated to purify it.

Two companies are engaged in the manufacture of wheat starch in the United States. Both use flour as a raw material, usually a flour milled from soft wheat. Both are interested in the manufacture of derived products from gluten (8).

Newer Methods. Considerable experimentation has been and is being done to overcome the major difficulties of all processes in the manufacture of wheat starch; *i.e.*, removal of the gluten. In every case, in the older processes, this operation has been laborious, time-consuming, and when fermentation is involved, wasteful of the raw products. Reports of progress in the literature are meagre.

From a consideration of the older processes it would seem logical to assume, inasmuch as the recovery of pure starch is facilitated by a limited fermentation, that a large share of the benefits derived are in conditioning the gluten; that is, changing its colloidal characteristics. It would seem that extended degradation of the gluten to bodies of low molecular weight is not an important or even necessary part of the process. It might be supposed that this desired modification in properties of the gluten could be accomplished by chemical treatment, possibly during a preliminary steeping operation. In that event, it might be possible to mill the grains and separate the components by procedures more comparable to those used in the manufacture of corn starch with high yields of products of first quality.

These considerations may have guided the development of a process recently suggested by the Northern Regional Laboratory (9). It is believed that by a pretreatment of wheat with sulfurous acid at lower temperatures a treated grain results which can be milled in equipment and by processes essentially as described for the production of corn starch.

The wheat is steeped at 100° F. for about 24 hrs. with a relatively high concentration of sulfur dioxide. An SO₂ concentration of 0.3 to 0.5% is the recommended range for the steeping liquor. About 8.5 gals. of this acid are used to steep each bushel of wheat. After the wheat is steeped and the steep water drained off, the wheat is ground, preferably in a stone disintegrator of the Buhr mill type rather than in the Fuss mill used in the initial milling stage for corn grain. It would appear that the gluten is sufficiently "granular" in characteristics to be passed over 26 mesh copper reels, No. 17 silk shakers, and tables without causing a slime to develop on the sieves or without settling with the

starch on the tables. The coarse material left on the reels and shakers is reground and again resieved. Tabling is quite satisfactory, at least with mill liquor as high as 3-4° Bé.

The gluten flowing from the ends of the tables may be recovered by settling and filter-pressing. It remains, however, in a somewhat altered physical state which may restrict its use for such purposes as those for which native wheat gluten is employed; *e.g.*, addition of wheat flour to raise the protein content of the latter above normal.

A 55 to 60% yield of starch with a protein content of 0.2% can be obtained by such a procedure on a pilot plant scale. The gluten fraction contains about 30% protein and is relatively high in starch. But further quantities of starch are readily recovered by washing the gluten after it is collected from the ends of the starch tables.

2. White Potato Starch. Although potato starch occupies a very important position in European markets, its importance in the United States decreased as the manufacture of corn starch was developed and extended. During the first half of the nineteenth century, a substantial portion of starch produced in the United States was made from potatoes. Even as late as 1880, there were more than 150 factories producing potato starch located throughout New England, the central, and midwestern states. A recent count showed that there are less than thirty plants, many of which are idle during some seasons. They are mostly located in Maine and are operated or owned by persons or corporations interested primarily in the sale of starch. For example, ten of twenty-four Maine plants are controlled by a subsidiary of a large corn starch manufacturing company (10).

Most of the factories manufacturing potato starch in the United States are relatively small and are equipped with simple machinery. Since acquisition by the larger starch-producing companies, several of these have been enlarged and improved with equipment of very modern design. Several new plants have been built recently. The annual capacity of all United States mills is 40 million lbs. The average annual production is less than half of this and actually was 15 million lbs. for the years 1925 to 1940.

The tubers of the potato plant (*Solanum tuberosum*) consist essentially of a brownish colored skin which surrounds a substance floury in nature when dry and which has a yellowish white color. The latter, in its natural state, however, consists of large cells which contain a high proportion of cellular fluid in which the starch granules are suspended. The external membranes of these cells are of a cellulose nature. The fluid consists of a water solution of soluble proteins, acids, and various inorganic salts such as phosphates of potassium. The average water content of the whole tuber is consequently quite high and varies between 65 and 80%. The actual starch content of the potato is, therefore, quite low, being about 10 to 25%.

The skin of the potato consists of a cellulose-like material, akin to wood in some respects. It is commonly referred to as cork substance.

The composition of the potato varies considerably, according to variety. For this reason, much work has been done in Europe to cultivate potatoes for starch manufacture. Among other things desired in a potato for this industrial use are (a) high yield of tubers per acre, (b) high starch content in the tubers, (c) thin skin, (d) low fiber content, and (e) low protein content. All of these factors must be balanced for the economical production of potato starch. Of course, the most important factors are the yield of potatoes per acre and the starch content of potatoes, the product of which is the possible yield of starch per acre. In general, the following may be taken as the average composition of Maine potatoes: water 70 to 80%, protein 2%, fat 0.1%, ash 1.0%, cellulose 0.5%, sugars 0.4%, and starch 10 to 30%. Organic acids are present such as succinic, tartaric, citric, and ascorbic. There are amino acids such as asparagine and tyrosine, the latter being a source of much trouble in starch factories due to its reaction with iron. Process water should be iron-free. Solanine is present during sprouting. Freezing of potatoes, induces a change of some starch to sugar and soluble products, which action reduces the yield of starch. Active enzymes present in potatoes, such as tyrosinase, cause color reactions to develop during processing and thereby give an off color to the milled starch. These enzymes are oxidases; therefore modern processes are designed to keep the pulp from contact with the air or to use a reducing agent such as sulfur dioxide in the process waters. In one process, for example (11), air is excluded from the time the potatoes are pulped until the pulp is freed of water in the centrifuges.

In the United States, where potato starch constitutes but a small percentage of the total starch made, it is not economical to grow potatoes for the purpose of starch manufacture. In normal times cull potatoes are used for processing. These consist of small, broken, misshapen, or otherwise unmarketable potatoes. Those that have been frozen may also be used, but as stated above, yields are lower. In years when abnormally large crops are produced and a resulting surplus leads to unfavorable market prices, marketable grades may also be used. Up to the present, however, an average of not more than 1% of the total crop of the United States is used for starch production. Potatoes used for starch manufacture in the United States contain from 14 to 17% starch and the yield obtained by the factories averages from 10 to 12% of the weight of the potatoes. The average price paid is probably not more than 10 cents per bushel (10).

The description of processes will be limited to more modern plants, where, under the guidance of competent engineers, 95% of the starch available is recovered (12).

Potatoes are delivered by trucks from nearby farms to hoppers which feed two washers in series. The first washer is equipped with a barrel-drum section where the cleaning action is induced by a centrifugal motion of the potatoes against the inner wall. Attached is a section in which heavy hardwood paddles force the potatoes, counter-current to a stream of water, to the exit end of the washer. They are removed from the first washer by conveyer to a second washer

equipped only with paddles for moving the potatoes and with water sprays for washing.

The washed potatoes pass to a hopper which feeds a Jahn, saw-tooth rasp. The rasped pulp is passed through rotary sieves, or reels, which remove the coarse particles. The coarser material is repulped. The fine pulp flows to a tank into which sulfur dioxide is introduced in the proportion of $\frac{1}{2}$ lb. per ton of starch present. The gas is introduced by means of a rotameter.

The pulp is then centrifuged in a continuous, imperforate, conical bowl type centrifuge, with a spiral ribbon to remove the sedimented solids. It is called a Uhland protein-water separator. The solids are resuspended in fresh water or process water containing but little soluble matter. The centrifugate is discarded.

The suspension passes over a series of fine sieves. The remaining pulp is ground in an abrasive stone mill, after which process it passes over two shaker sieves for washing. The final pulp is discarded.

All starch milk then flows to a second Uhland protein-water separator. The solids are suspended in fresh water and pass to starch-settling tables.

The starch is flushed from the tables by jets of water at high pressure to a storage tank which feeds silk shaker sieves. The silk is about 200 mesh. After passing these sieves, the starch is freed from water in centrifuges. The filtrate passes to the first protein-water separator. The starch cake is conveyed by a spiral conveyer to a bucket elevator which feeds the drier, after which it is pulverized in a hammer mill, bolted, and packed.

Other modern equipment in one mill includes a rotary brush sieve for the first grinding from the rasps, and a large, rotary, vacuum filter for final removal of water while the filter feeds a continuous belt drier.

The starch produced in our modernized factories compares favorably in quality with the best that has been produced in Europe.

In respect to the general manufacture of potato starch, it is interesting to note that Wiegel (13) reports that the velocity of sedimentation is increased for potato starch by the addition of an acid; *e.g.*, sulfurous acid. In a previous chapter, the effect of electrolyte concentration on the sedimentation of corn starch has been discussed. It is believed that the experiments reported by Wiegel are, mainly, another illustration of the same phenomenon and the effects are not due entirely to either hydrogen ion activity alone or to the specificity of any particular anion. That the velocity of sedimentation of starch is increased by electrolyte concentration is very easily demonstrated. If a sample of starch is thoroughly washed repeatedly in distilled water, a suspension of it prepared and poured into two cylinders, and a few drops of a strong solution of sodium chloride stirred into one cylinder, it will be observed that practically all of the starch in the latter sample settles out at a relatively fast rate, whereas in the sample containing no salt the smaller granules, at least, settle at a materially slower rate.

In some manufacturing processes, the potato starch may be treated in addition with limited amounts of oxidizing agents such as permanganate, hypochlorite, and ozone to remove certain impurities, particularly those which impart a

characteristic odor and taste to the starch. Such impurities are not necessarily objectionable when the product is used without additional modification and the odor and taste characteristics that have developed in the native starch are quite effectively corrected by the reagents mentioned to produce a satisfactory edible product. However, when the starch is dextrinized, as in the manufacture of gums that are remoistened to make adhesives for envelopes, labels, stamps, and stickers, small traces of these potato starch impurities are sufficient, with the effect of heat used in the dextrinization, to make a rather objectionable product. Even starches that have been treated with reagents are not entirely satisfactory for this purpose. The author has found that the characteristic, unpleasant taste which develops in the manufacture of remoistening gums from potato starch is prevented by pretreatment of a water suspension of potato starch with gaseous chlorine. The operation may be performed in accordance with a method given for treating starch described by Kerr (14). This is done by introducing about 1.2 lbs. of chlorine gas per 100 gals. of starch slurry of 22° Bé. and holding it for 2 to 3 hrs. at room temperature after contact with the chlorine but before an antichlor is added to remove the excess of reagent. The starch is then neutralized, washed, and filtered. This pretreatment also appears to facilitate the subsequent dextrinization.

3. Sweet Potato Starch. Sweet potatoes have been used as a source of starch for over a century, particularly in Japan. Indeed, it has been estimated (15) that about 30% of the starch production of that country has been sweet potato starch in recent years. Experimental work on the production of this starch was started in this country during the last part of the nineteenth century, and at least one attempt was made to produce it commercially in our southern states prior to 1934. At this time, government funds were appropriated to finance the operation of a sweet potato starch plant at Laurel, Mississippi. In addition to the primary object of aid for southern farmers and laborers, a secondary object was to supply a part of the domestic demand for root starches by extraction of starch from the roots of the sweet potato plant.

Early experimental work has been reported by Hardin (16), Shiver (17, 18), McDonnell (19), and Keitt (20). More recently the United States Bureau of Soils and the United States Bureau of Agricultural Chemistry and Engineering have carried on the research to develop economic methods of manufacture. Reference is made to the reports of Balch and Paine (21) and of Thurber (22, 23) in tracing the development of methods, and to the report of Paine and coworkers (24) which summarizes production methods and other data on the subject for the Laurel, Mississippi, plant.

As a source of starch, the sweet potato (*Impomoea batata*) compares favorably with white potatoes. The starch content varies from about 14 to 28%, depending on the variety. A bushel of sweet potatoes, about 60 lbs. when harvested, yields from 10 to 12 lbs. of starch.

Except for methods used for the removal of color, the manufacturing process for sweet potatoes is quite simple and comparable to that for other root starches.

Sweet potatoes deteriorate rapidly after being harvested and must either be processed immediately or dehydrated. Dehydration by heat entails an added expense to that of a raw material of relatively high cost (about 30 cents per bushel for the grade of potatoes used for starch, as harvested, normally (15). Starch production has therefore been seasonal heretofore, and laborers from the farms producing the potatoes are employed for the manufacture of starch.

Potatoes from nearby farms are delivered to temporary storage bins from whence they are taken by water flumes to soaking pits. These pits contain a horizontal, slowly moving spiral and potato lifter. After the adhering clay and mud has been loosened, the potatoes pass to a rotary washer. These are rotary drums about 4 ft. in diameter and 20 ft. long, with high pressure water sprays for removing the dirt.

The washed potatoes are transported by a bucket elevator to a hopper feeding the first pulping mill. Hammer mills are used for this operation instead of the rasps used in the manufacture of white potato starch. Here, with the addition of process water, the potatoes are coarsely pulped and passed on to a system of haker screens, where the loose starch is removed. The pulp is then reground in a second hammer mill, and liberation of the starch is completed.

By the use of counter-current process water, the product of the second grinding is passed first over shaker screens, where the last of the starch is washed out, then to reels, to partially remove water from the pulp, and finally to a continuous roller type press. In the latter, the rolls are forced against a filtering drum, 5 to 6 ft. in diameter and 2 ft. wide, by a hydraulic pressure mechanism. The pressed pulp is delivered to a rotary drier, heated counter-currently by steam heat.

The starch slurries are collected and passed over screens to remove the last of the larger fiber particles, and then are tabled. Tabling is accomplished in two operations, the last being done with fresh water. There is a 2 hr. lag period between screening and tabling. This is brought about by the use of a large reserve tank which supplies the first tables. Such a lag is thought to be desirable in removing color from the starch, when the conditions of treatment to be described presently are used.

Tables are operated at a pH of about 9 (8.6 to 9.2) because of the alkaline process used. The density of the table liquors is held to about 5° Bé. The flow rate is 3 to 5 gals. per min. over 110 ft. concrete tables, inclined 1/32 in. per foot. Constant feed to the tables is maintained. A constant head of starch is circulated through a header line to feed lines. The feed line consists of a 1 in. pipe, extending vertically, at least 3 ft. from the header to within a few inches above the bed of the table. A quick opening valve is installed in the feed line, close to the header.

The density of the first tailing water is about 1.2° Bé., from which some starch may be recovered. The second table; tailing water is about 0.5° Bé. Starch in tailing water is recovered by use of cone bottom settlers, with an 8 hr. cycle, from which the concentrate is passed to two imperforate basket

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The density of the first tailing water is about 1.2° Bé., from which some starch may be recovered. The second table, tailing water is about 0.5° Bé. Starch in tailing water is recovered by use of cone bottom settlers, with an 18 hr. cycle, from which the concentrate is passed to two imperforate basket

centrifuges. The recovered starch is blended with starch from the first table and passes to the second tables.

Second table starch is flushed off the tables with fresh water at 10–15° passed over a 200 mesh screen, and pumped to a tank. A 2 hr. treatment with a slight excess of hypochlorite is given the starch at this point. After the treatment, the residual chlorine is eliminated with sulfur dioxide. The starch is adjusted to the desired pH and is then centrifuged free from water. The centrifuges operate at 1200 R.P.M. with a 40 in. basket. The starch leaves the centrifuges with about a 35% moisture content and is dried to 12% moisture in a batch type, vacuum drier. The dry starch is pulverized and screened on bolting silk.

The wet process is alkaline throughout. Clear, saturated calcium hydroxide solution is introduced at the shaker screens, and then travels counter-current back to the first grind mills. Additional lime water is added at this point as necessary, at the tables to maintain the proper pH. The process depends on a minimum of delays; otherwise microorganisms may become active, and the pH may drop off, in which case more lime water is added.

It is claimed that the function of calcium hydroxide is to solubilize certain pigments, to flocculate certain undesirable gums so that they may be removed with the pulp, and to produce a pulp of favorable consistency.

An acid (SO_2) process has been experimented with, but precipitation of impurities resulted during table separation. Moreover, excessive corrosive action was noted, which necessitated an alteration in equipment.

The control of microorganisms has been attempted with the alkaline sodium sulfite, but it is reported to give disappointing results. Frequent stoppage of the screens resulted, caused by coalescing of the sweet potato "latex," which hardened on the surfaces of the screens. The development of mold filaments was noted, which interfered with sieving. Moreover, the pulp became undehydrated as it passed from screen to screen and finally became very difficult to free from water with a roller press. The sulfite process has been described by Thurber (22, 25). Balch and Paine (21) have described an acid sulfite process followed by an extraction of pigments with alkali. With the usual iron equipment used in milling, however, this method produced a starch colored by precipitated iron oxide. The calcium hydroxide process, used at the Laurel sweet potato starch plant, was suggested by Richee (24).

It is interesting to note that by the use of the latter process the sedimentation rate of starch is not greatly different from that of the impurities suspended in the table liquors. Hence the use of sedimentation tanks to effect a separation and possibly also certain continuous hydroseparators is not entirely satisfactory (26). Yet surprisingly enough, by the proper adjustment of the velocity of flow over suitable tables, rather satisfactory sedimentation of starch results at 5° Bé.

It is also interesting to note that the use of electrical, vibrating screens was tried in experimental runs at Laurel, but was abandoned because of an anticipated low capacity.

With freshly dug potatoes, factory operation is limited to about 100 days, beginning the first part of September. Not more than a 3 to 5 day supply of potatoes is maintained in southern climates, owing to rapid changes that develop within the sweet potato after digging. Research has therefore been concerned with dehydration of sweet potatoes for storage and subsequent milling. The usual curing process is not feasible for preparing potatoes for storage for starch manufacture, owing to the high amylase content. Hopkins and Phillips (27, 28) have found, however, that when ground or sliced potatoes are treated with SO_2 vapors, the membranes become permeable enough so that a large portion of the juice can be pressed out by mechanical means, which of course is much cheaper than total dehydration by heat. As high as 70% of the natural juice has been expressed by such a process. Flue gases are used to dehydrate the pulp to 12% moisture content, at which it is stable for storage.

It is reported (12) that an additional improvement can be made in the dewatering process by the addition of a very small proportion of dry, hydrated lime.

The expressed juice consists of sucrose and other carbohydrates of low molecular weight and has a dry solids content of 9.5 to 11.5%. It would appear suitable as a base for certain fermentations or for concentration to a molasses.

Experiments reported in progress are a simplification in the process for the manufacture of sweet potato starch, based on the elimination of a greater part of the soluble material in the pretreatment described.

In addition to the development of the manufacture of sweet potato starch at Laurel, a second plant is reported to have been built in the south, at St. Francisville, Louisiana, in 1939 (12).

4. Arrowroot Starch. The manufacture of arrowroot starch is quite similar to the manufacture of tapioca starch, each step in the manufacture of the latter having its counterpart in the manufacture of the former. Indeed, so similar are the two starches in respect to source, manufacture, and paste characteristics, they are often confused and much of the arrowroot of commerce is actually a variety of tapioca. In the older literature, tapioca was often referred to as Brazilian arrowroot.

Arrowroot starch is, none the less, a distinct variety of starch, the manufacture of which varies slightly from tapioca in one or two respects, which will be discussed. The starch is obtained from the roots of the *Maranta* plant (*Maranta arundinacea*, L.). The plant belongs to the family Scitamineas, genus *Maranta*. It is a herbaceous, rhizomatous plant, having stems about a foot and a half high bearing oval lanceolate leaves, oval-shaped flowers, white in color and arranged in twin clusters, which produce small red seeds. The root, where the aerial stem starts, is elongated, somewhat flattened, and pointed. It is covered with regular scales at each section, under which scales are the germs by which the plant may be propagated (as well as from the seeds). The scales, if not completely removed, impart a bitter taste to the starch. The roots contain about 25% starch and 65 to 70% water, but due to the very tough nature of the cell walls

enclosing the starch, industrial yields rarely exceed 15% except in the most efficiently operated mills.

Hence the essential differences in manufacture, compared to tapioca, are complete removal of the scales and the maceration stage, which requires the use of a series of mills with saw-like blades to tear the cells apart and hammers to convert the pulp to a paste. From the mills, the paste is passed into a reel and the starch is washed away by fine sprays of water. The washed pulp is reground at least two additional times in more efficient processes.

Even in the more progressive mills, considerable manual labor is involved in the various processes, which may account for a part, at least, of the higher market price of the starch. Equipment in the larger mills in the British West Indies is, none the less, very modern and comparatively efficient.

Drying the starch in the final stages of production is done at low temperature in driers of the Thomas heater type, in the larger mills. In these driers, endless belts carry the starch to regions of increasing temperatures, the starch finally attaining a maximum temperature of about 55–60° C. Some mills use rotary driers and some use specially designed vacuum ovens.

5. Sago Starch. Sago starch is a name applied to a great variety of starches which are derived from the pith of various palms. Among those that have been used are *Cycas circinalis*, *Cycas revoluta*, *Sagus rumphii*, *Sagus farinifera*, *Convolvulus batatas*, and *Areca oleracea*. All of the starches are similar in some respects. They are quite large individual granules which occur, naturally, in a fair state of purity. Only the most elementary equipment is needed for preparing the starch.

Considerable sago starch is made from *Cycas circinalis*, a palm fern which grows with particular luxuriance in the marshy lowlands of the East Indian archipelago. These palms grow up to 30 ft. in height and 2 ft. in diameter. They are cut about the sixth year of their growth, however, for starch manufacture.

The stems of the palm have a comparatively thin, woody exterior, which is easily removed by splitting the stems lengthwise. The central portion, consisting of a very loose pith, is removed and cut up into small portions.

Manufacture consists of kneading these pithy particles in a current of water. The water is run over a sieve to remove extraneous pith and then into a series of troughs in which the starch settles out. The starch is rewashed, dehydrated, ground, and bolted, when it is to be sold in the form of powdered starch.

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CHAPTER VI

EVALUATION OF MODIFIED STARCHES IN PRACTICE

1. Viscosity of Hot Pastes.

A. General Considerations—The principal uses of modified starches are as a sol, paste, or gel. Therefore, the principal characteristics of a modified starch are its colloidal paste properties such as viscosity, plasticity, gel strength, rigidity, and similar characteristics. In practice, the modification of a starch is usually followed by some viscometric measurement, and the modification is carried to the point when the desired level of viscosity has been attained. After manufacture, however, the product is usually observed in supplementary tests made as a check on the perfection of the manufacturing process. The selection of supplementary tests is guided by a consideration of the use to which the starch will be put; *e.g.* by tests of the gel strength of starch used in the manufacture of gum drops, by cold paste body and plasticity measurements of starch designed for use in the clay coating of paper, and so forth.

Although the terms viscosity and fluidity of starch pastes are spoken of in industrial practice, most starch pastes do not possess viscosity in the sense in which the term is applied to the more perfect fluid bodies, such as water, but rather in that they possess an anomalous viscosity. The effect measured is usually the result of a combination of many inherent properties of starch that has been gelatinized in aqueous media. A fundamental discussion of this subject is given elsewhere in this text. In a review of methods it should be recalled that vis-

cosity measurements are complicated principally by plasticity and by elastic effects which arise from residual structural units of the starch granules or new structural units in the paste which form through associative forces exhibited by the molecules or colloidal aggregates. The methods of measuring viscosity and the instruments employed, therefore, should be so chosen as to eliminate gross misconceptions as to the viscosity of a paste. In any event it will be appreciated that a statement of the viscosity of a starch product is rather meaningless in industrial practice without a supplementary determination which will throw some light on the additional properties of plasticity, tendency to gel, and other phenomena of the cold paste. Above all, it should constantly be borne in mind that many starch pastes are unstable in the colloidal sense and that the elements of structure which are found in these pastes are unstable. Because of this they are, in many cases, thixotropic; that is, their viscosity is abnormally depressed by increasing the applied stress.

Associative forces in starch paste are relatively slow in exerting their effects. Among other things, the presence of colloidal structures within a paste depends upon the extent to which the starch is gelatinized, the extent of dispersion, the pH and dilution of the paste, and the temperature to which it is brought to make the observation and measurement. It should be obvious, therefore, that the determination of even a relative, apparent viscosity of starch is no simple matter. To obtain reproducible results, even with suitable methods, requires the most rigid adherence to a prescribed technique for preparing the starch sample and the paste and for performing the test itself.

From a consideration of the chemical and physical properties of the starches, described elsewhere, it should be apparent that a more reliable estimate of the viscosity of starch paste would be obtained if observations were made at temperatures, pH values, and dilutions at which associative forces are at a minimum. Such conditions, however, would render the result of little practical significance. For routine testing, compromises are made in establishing the conditions of test. It is the more common practice, therefore, to make primary tests for viscosity at some dilution intermediate between those used in the industrial application and those high dilutions at which the results obtained are of academic interest only. Unless specifically stated otherwise, the pH is left in the range which is considered normal for starch. The determinations are, in general, run at elevated temperatures at which retrogradation effects are at a minimum. When it is obvious that the results so obtained would be misleading for a particular industrial use, the primary test is supplemented with a second test; for example, when the starch is to be applied as a cold, aged paste, a second determination is made after a definite cooling and aging period. It is appreciated that the latter test may be materially affected by retrogradation and gelling effects and hence is not termed viscosity but rather cold paste body.

A test which involves a more perfect measurement of viscosity has been proposed and used in practice in the past. The starch is dissolved at low concentration in dilute sodium hydroxide and the specific viscosity of the solution is,

observed after a definite period of time, at a fixed temperature. Very poor correlation results, however, when these viscosity values of starches from different sources, those obtained by different methods of manufacture, and starches from different sources prepared by different methods are compared with the working properties of the starch in practice. Use of the test assumes complete gelatinization and dispersion or solution. This rarely, if ever, is attained in practice. Furthermore, it will be observed that different starches (compare an acid-modified corn starch with a chlorinated starch, for example) show different rates of degeneration in alkaline solution. Quite naturally, substances which are effective buffers in alkaline media, and which are often found in starch products, will tend to give the starch an abnormally high viscosity (or low fluidity) by this method.

A complete review of the literature covering methods which have been used to evaluate a starch must, of necessity, be unduly long, because at one time or another almost every method that has been proposed to measure viscosity has been applied to starch. Such a review would be unwarranted, for many investigators reporting the use of these methods have failed to appreciate the anomalous viscosity of starches. When sound conditions for performing a particular test are developed (which is the general rule) the conditions imposed limit the practical significance of the results.

The industry has, therefore, empirically developed several procedures in which a variety of equipment is used, and these tests have apparently served their purpose admirably well. These viscosity tests fall into three main groups, as indicated above: hot paste viscosity, cold paste body, and the viscosity of an alkaline solution of the starch, the so called alkaline fluidity test.

B. Scott Test for Hot Paste Viscosity—Probably the most widely used method for determining the hot paste viscosity of starch pastes is to allow the paste to fall freely from a funnel, pipette, or other vessel with a restricting orifice. The Scott test is one of such methods. This type of equipment was first built for the petroleum industry. MacNider (1) suggested its use for starch pastes. Various modifications of the method have been introduced and are described in the literature (2).

The essentials of the method follow. A quantity of starch at known pH is stirred to a slurry with 280 cc. of distilled water in a German silver beaker. The beaker and contents are then placed in a boiling water bath, in which, with stirring, the starch is gelatinized and heated for 15 min. At some definite period of time prior to 15 min., 200 cc. of the paste are transferred to a Scott viscosity cup, also in the boiling water bath. Usually 2 min. are allowed for the paste to stand in the Scott cup. At the end of a total elapsed heating time of 15 minutes, the plunger valve which closes the orifice on the bottom of the cup is raised and the time in seconds is noted for a given volume of the paste to fall into a graduated cylinder. The time, in seconds required, is the specific Scott test viscosity.

This viscosity may also be considered as a relative viscosity, but not relative to water. It is relative in the sense that standard starches are used to set up permissible limits of variation in the viscosity of other starches to be tested by this method.

The same amount of water is used in making up the starch slurry for testing all starches. The actual weight of starch used is varied, depending on the range of viscosity of the particular starch to be tested. The weight of starch employed varies between 10 and 100 g. The moisture of the starch is taken into account, and the results are stated either on a dry starch basis or as the viscosity of a given weight of starch at a known moisture content, whichever method is preferred for reporting the results. After the paste is tested, the pH is measured and, if it falls outside of the range anticipated, the test is repeated, the pH of the starch being corrected accordingly.

The boiling water bath should be of sufficient capacity to prevent overheating and slight variations in temperature due to convection currents and radiation. An acceptable method of heating is to blow steam at low pressure into condensate or distilled water. Multiple steam jets are evenly distributed over the bottom of the bath. An overflow and several free outlets or stacks are attached to the bath to provide a ready and continuous removal of excess steam and water. The water should boil vigorously. For comparative purposes, within a laboratory, no correction is applied for different elevations or other variables affecting the barometric pressure.

In routine testing, stirring of the starch pastes is mechanical and continuous. Smooth paddles with rounded edges, geared to a motor at constant speed, stir the paste. The stirring motion is rotational at 200 R.P.M. The paddles are deflected so that paste is moved from the outside walls of the beaker to the central axis. The beaker is then covered to prevent evaporation. In modern practice the Scott cup supplied for general viscosity measurements is modified for starch determinations. The cup is supplied with a wide aperture for overflow so that an excess of starch paste may be poured quickly into the cup. During the 2 min. that the paste stands in the cup, its volume is automatically adjusted to 200 cc. The orifice of the cup is carefully adjusted, the starch used being one whose viscosity according to the Scott test is known. The latter is determined in a master cup which should be used for standardization work only. Normal, unmodified corn starch manufactured currently requires 90 to 100 sec. for the first 50 cc. of paste to pass the orifice of the Scott cup; the paste which is made up of 15 g. of starch (containing 12% moisture) and 280 cc. of added water normally has a pH of 5.0 to 5.5. Fig. 46 shows a Scott test assembly and a second cup to show its overflow tube.

The amount of paste collected in the test, if preferred, may be any convenient volume. This volume should not, however, be unduly large. In any event, the head of paste in the cup will decrease in proportion to the amount of starch which is allowed to flow from the cup. The change in pressure head is reflected in a change of the rate of shear. Viscosity values of starches are closely related

to rate of shear, and, unfortunately, this relationship is neither linear nor necessarily the same for all types of starches. However, for comparative purposes, variation in pressure head may not be a serious disadvantage in the use of the Scott test or other instruments for measuring viscosity by transpiration methods, provided this variable is kept within restricted limits. Increasing the volume of paste collected in order to obtain significant figures for very thin starches should not be carried too far. Instead, it is the more common practice to increase the amount of thin boiling starch used in the test.

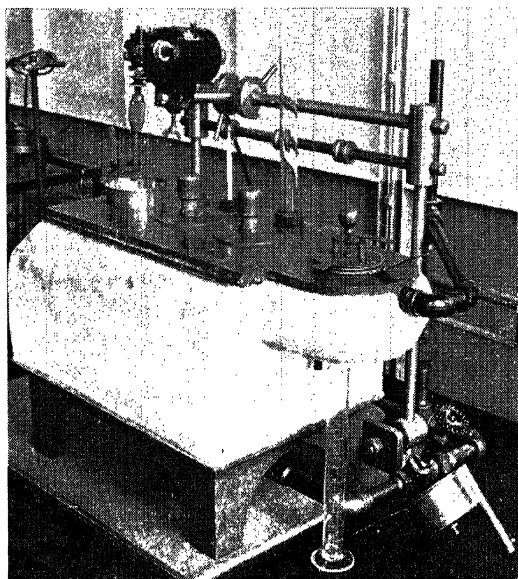


FIG. 46. Scott test apparatus for measuring the hot paste viscosity of starch.
Photograph by H. Meisel.

As with other tests for starch paste viscosity, absolute cleanliness of equipment is essential. A precision balance should be used, and all operations should be handled in accordance with quantitative practice.

C. Soybolt, Hot Paste Viscosity—Another instrument of the general type described above is the Soybolt, which is preferred to the Scott viscosimeter by some laboratories. The same precautions are to be observed in making a determination with the Soybolt instrument as outlined above for the Scott test.

D. Dudley, Hot Paste Viscosity—The Dudley pipette is frequently used to measure hot paste viscosities. Its use is limited, however, to comparatively thin pastes. It is, therefore, used more often on starches modified to a rather considerable degree.

The method involved is rather elementary and consists in drawing, by suction, a given volume of paste into a calibrated pipette at a definite temperature and immediately noting the number of seconds for the pipette to deliver its charge of paste into an open vessel. Normally, no provision is made for keeping the paste at constant temperature during the test, and such a procedure would, of course, rule out the use of the pipette for higher temperatures. The pipette may, of course, be jacketed for determinations at high temperature.

The method probably has its greatest use as a supplementary test in industrial modifications of starch. It may be used to follow the enzymic liquefaction of a starch in practice, *e.g.* the action of diastase up to 70° C., and to check the anticipated extent of such liquefaction before application of the product at some elevated temperature, *e.g.* 50° C.

Dudley pipettes are calibrated, water being used as a standard.

E. Other Tests for Hot Paste Viscosity—Other types of similar equipment either of special design or standard equipment are also used. The Redwood viscosimeter appears to have been used in England (3), whereas the Engler (4) is extensively used in Germany. It might be remarked at this point that the United States Bureau of Engraving and Printing specifications for certain modifications of starch specify viscosity by Engler.

Another modification used in supplementary tests should be mentioned. This involves determining the viscosity, by any suitable method, of a paste prepared by cooking starch with open steam jets. It is common practice in the industrial application of starch to prepare the paste with live steam as a source of heat rather than by an indirect application of heat such as a jacketed kettle. As might be anticipated from a consideration of the fragile nature of starch, the viscosity of a starch paste, as well as other colloidal characteristics which are a function of the extent to which a starch is dispersed, may be quite different when the paste is prepared by being brought to and held at a given temperature with live steam than when a similarly made paste is prepared by indirect heating. When this modification in cooking is used, care should be exercised that proper agitation or stirring is provided either by the proper arrangement of the steam jets in the cooking vessel or by supplying additional mechanical stirring. Obviously the steam used should be dried and corrections made for the steam condensation, for the evaporation of moisture during the cooking period, or for both.

2. Cold Paste Body.

A. Stormer, Cold Paste Body—When an evaluation of cold paste body is desired, the Stormer viscosimeter may be used to good advantage. Alsberg and Rask (5), Kerr (6), and others have used this type of instrument. It is comprised of a cylinder immersed in the test paste which is contained in a metal cup surrounded by a water bath. The immersed cylinder is rotated by a free-falling weight acting through a gear and pulley system. The time in seconds necessary for a given weight to produce a certain number of revolutions is taken as a measure of cold paste body. A revolution counter is a part of the instrument.

Details for applying the method follow. The starch is gelatinized in the same manner as recommended for performing the Scott viscosity test. Heating is continued for the full 15 min. period without transfer of the paste to the Scott viscosity cup. Instead, the paste is placed immediately in a constant temperature water bath, preferably 25° C., in a closed container. At the end of a definite aging period, any surface skin on the paste is carefully removed and discarded. The remaining paste is very gently stirred with a spatula for several seconds, whereupon it is transferred to the cup of the Stormer viscosimeter, and the viscosity is noted at 25° C.

The weight of starch used in making up the paste should vary in a fixed manner with the range of cold paste body of the starch. For unmodified corn starch, 10 g. plus 280 cc. of water make a suitable ratio. For the thinner, acid-modified starches, a ratio of 15 g. to 280 cc. of water is recommended. In any event the dilution should not be so high that the results lose practical significance. On the other hand, the concentration of starch should not be so high that the paste will gel when cooled. Even for rather plastic pastes, incorrect results may be obtained because of the tendency of the cylinder to cut through "short" pastes or to wind up viscid pastes around the shaft of the rotating cylinder.

Cold paste body depends upon the extent of gelatinization, the degree of dispersion, the temperature and length of heating, the speed of cooling, the minimum temperature to which the paste is cooled, the length of the aging period at a definite temperature, agitation during cooling or aging, pH of the paste, and other variables. All operations for preparing the final paste for test must, therefore, be most carefully standardized. In routine tests, observations are made after 3 hrs. aging at 25° C. and occasionally on a duplicate sample after 24 hrs.

Each type of modified starch requires adjustment for the weight used to actuate the cylinder in the Stormer viscosimeter and the number of revolutions counted. If too heavy a weight is used, so that the speed of the cylinder is too fast, an error results from turbulence in the paste. If the weight is too great and the number of revolutions counted is excessive, the result is complicated by the thixotropic nature of starch pastes. In most cases the first revolutions counted will be found to require a substantially longer time than the later revolutions. Although this effect is difficult to eliminate completely, it should not be exaggerated. If the weight used is too small, and particularly if a great many revolutions are counted, the reverse effect may be found. The paste may appear to become thicker owing to retrogradation or gelling tendencies exerting their influence during the period of test. A balance between the two opposing effects would, of course, represent ideal conditions. For unmodified corn starch pastes, made as described above, a 75 g. weight is used, and the time in seconds for 50 revolutions is taken as the Stormer test for cold paste body. For the medium range of acid-modified starches, 15 g. of starch are used in the test and a 50 g. weight and 50 revolutions are satisfactory.

B. MacMichael Viscosimeter Tests—The instrument introduced by MacMichael (7) has been applied to the determination of starch viscosity by Gallay

and Bell (8) and others. The instrument may be used for cold paste body. This instrument consists of a cylindrical cup, in which the starch paste is placed, surrounded by a bath with an electric heating device to regulate the temperature. The cup and bath rotate at a constant speed which may, however, be regulated over a wide working range. A second cylinder is suspended in the paste by means of a torsion wire. The twist that develops in the torsion wire is read by noting the position of a fixed pointer in relation to a moving scale mounted on the torsion wire. The instrument is supplied with several interchangeable torsion wires so as to permit measurements over a wide range of viscosities.

Owing to the constantly applied shearing stress throughout the determination, most starch pastes exhibit a constantly falling viscosity. Especially for unmodified corn starch, one is in doubt as to how long to wait before making the observation or whether to take a reading the instant the motor starts rotating the cup. The latter is obviously too high in that the plasticity factor is overemphasized. Yet, if an interval is allowed before an observation is taken, one cannot be sure that other starches will show the same rate of decrease in the apparent viscosity or cold paste body.

The instrument is applicable, however, in a limited way, to certain classes of modified starches. It is particularly useful in certain supplementary viscosity tests, mentioned at the beginning of this chapter. These tests involve application of the starch in a manner similar to that used in practice; *e.g.*, starch may be used to coat papers with clay in the manufacture of printing paper of high grade. The starch, therefore, is gelatinized and mixed with the clay in a definite manner comparable to that used in practice, and the viscosity of the mixture is determined in the MacMichael viscosimeter at 25° C. Various and significant differences will be observed in the colloidal behavior of the several types of modified starches tested by such a procedure. For such tests, the viscosity in degrees MacMichael may be observed at several different speeds of rotation. These values are plotted and, if the function is essentially linear, a specific plasticity, as well as the viscosity, may be determined from the data obtained. The plasticity is proportional to the value at which the extrapolated curve for viscosity intercepts the axis of the degrees MacMichael.

C. Hoeppler Viscosimeter for Viscosity Measurements—An instrument which involves the use of a different principle from those described above is the Hoeppler (9). A recent discussion of this instrument is given by Wobser and Müller (10). Measurements are made of the time required for a given weight to fall, in vertical motion, through a measured column of paste. The column is surrounded by a constant temperature bath so that with only slight modifications the instrument can be adapted to the measurement of hot paste viscosity as well as cold paste body. However, the instrument would seem to be more adapted to the precise measurement of the cold paste body of modified starches.

Various weights calibrated so that with fluid bodies the viscosity in centipoises may be readily estimated from the time required for the weight to fall through

the column are supplied. The weights are in the shape of spheres and are of such magnitude that a wide range of viscosities may be covered.

Pastes may be prepared by a standard procedure such as those outlined for the Scott and Stormer tests. As soon as it is prepared, the paste is transferred to the glass tube of the instrument. The sphere, selected from the six supplied by the maker, is placed in the tube after the tube is filled with the paste. A definite period is allowed for the paste to come to and stand at the temperature at which the observation is to be made. Constant temperature is maintained in the instrument by circulating water from a large constant temperature bath through a glass jacket surrounding the column of paste.

At the time of observation, the sphere is caused to fall through the paste several times by inverting the tube and attached water jacket, which are attached to a stand by a swivel joint. Finally, a 3 min. interval is allowed and the tube is again inverted and the time required for the sphere to fall from the upper mark on the column to the lower mark is noted. Several observations are made and the results averaged. 3 min. intervals are allowed between successive observations.

The choice of the sphere to be used is governed by the rate of fall, which should not exceed 1 cm. per second. Valenta (11) and others have pointed out the danger of the sphere not following a straight, centric path in instruments of this type. Hoeppler has inclined the tube to an angle of 10° in his viscosimeter. This is claimed to eliminate the tendency of the sphere to follow a spiral path in its fall. Constancy of the angle of inclination is assured by leveling the base and stand of the instrument with the aid of a built-in spirit level. Although the column may be rotated around an axis in order to reverse the direction of motion of the sphere, a set-screw is provided by which the position of the column can be fixed at the correct angle.

In instruments of this type it is essential that the sphere should be released under the surface of the test liquid and should be accompanied by a minimum of vibrational disturbances. The design of the Hoeppler instrument has reduced the end effects of the column to minimal proportions. The operator must exercise his ingenuity to reduce the effects of vibrational disturbance which may result from uneven temperatures. Too rapid cooling of the paste and running the test at too short an interval after placing the paste in the tube are sources of such error. After several preliminary inversions of the tube, it is essential that a sufficient interval be allowed for the movement of the contents to subside. A period of 3 min. is recommended for starch pastes. However, pastes from certain starches may require a longer period of rest to eliminate erratic results. In inverting the column care should be taken to avoid undue vibrational disturbances.

Some difficulty may be experienced with highly opaque pastes in observing the fall of the sphere.

It will be seen that there are certain obvious advantages in using an instrument of this type; *e.g.*, simplicity of operation, speed of setting up equipment and in

determination of the results, possibility of repeating determinations on the same sample, reduction of the personal factor to a minimum, and ease of standardization.

3. Pasting Characteristics of Starches or Paste History.

A. Caesar Consistometer—Certain viscosimeters may be used to measure several characteristics of starch simultaneously. The starch slurry is introduced into the apparatus, heat and agitation applied, and a record is obtained of the body of the starch over the entire period from the time gelatinization starts until the paste is cooled to a given temperature. The use of such instruments is becoming more of a practice since Caesar (12) directed attention to the value of obtaining a continuous history of the pasting operations. Such characteristics may be evaluated as (a) gelatinization point of the starch, (b) peak viscosity obtainable with a definite cooking procedure, (c) specific viscosity after a prescribed cooking operation, (d) rate of increase in paste body with falling temperature, (e) cold paste body after a time interval at a lower temperature. All of these characteristics are important to know when starch products are applied in industry.

The essentials of the method described by Caesar are to suspend a standard paddle, connected by a shaft to an electric motor, in a beaker containing the starch slurry. The latter is surrounded by a heating bath, and the temperature is raised at a regulated rate. After the starch is cooked, cold water may be introduced into the bath to cool the paste. The paddle and motor move at constant speeds. Changes in the viscosity or paste body are indicated by the differences in the electrical input to the motor to maintain a constant speed of stirring. To obtain significant differences in observed values, however, it is recommended that a relatively high ratio of starch to water be used in the test; for example, 20% starch. Radley (13) has used a similar type of instrument, except that the power input to the motor is kept constant and a variation in the speed is recorded.

B. Brookfield Viscosimeter—A commercial instrument known as the Brookfield viscosimeter is built on a slightly different principle. Spindles of various types are driven by a synchronous motor and the "drag" of the starch paste, acting on the spindle, is taken as a measure of the viscosity.

C. Comparometer—The author has used a similar type of instrument, which has been called the comparometer, in industrial work for many years. Two beakers containing starch slurries are heated together. The frameworks holding the beakers in the heating bath are both geared to the same motor, and identical rotating speeds and heating and cooling conditions are assured for both samples. A standard, industrial starch is placed in one beaker; the starch to be studied is placed in the other. Even minor variations between the two products may readily be shown by comparing the torques developed on the stirrers from the time the starches start to gelatinize until they are cooled to some definite temperature.

D. Brabender Amylograph—A more recent improvement in an instrument in which the same general principle as outlined above is used is the Brabender amylograph. A lengthy discussion of this comparatively complicated instrument is given by Müller (14). In this method, the paste is held in a cup surrounded by an air bath for temperature control. The cup is revolved at constant speed. The measuring device consists of a disc to which are attached several short rods extending into the paste. This mechanism serves as a stirring unit also. The

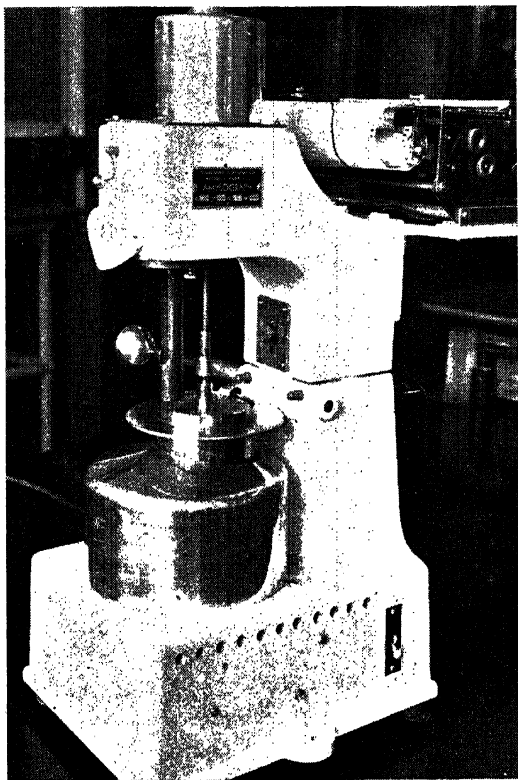


FIG. 47. Brabender amylograph. Photograph by H. Meisel

torque impressed on the measuring unit is transmitted to a recording torsion balance. A continuous, graphical record is traced by the instrument over the entire period of test. An elaborate temperature control is provided to increase the temperature of the starch at a fixed rate. The rise in temperature may be checked at any point and held at this level by a thermostatic control. Then, by switching off the heat supply, the paste may be cooled to any desired temperature and the change in viscosity noted. Fig. 47 illustrates the amylograph.

In addition to obtaining a permanent pasting record of a starch, such characteristics of a starch may be estimated from the graph as (a) gelatinization point, (b) rate of gelatinization, (c) peak viscosity, (d) viscosity after any specified heating period, and (e) congealing properties such as the rate and amount of increase in the viscosity with decrease in temperature.

The instrument appears suited for other supplementary tests. The manufacturer suggests that it may be used to study the liquefaction of starch by enzymes. It may also be used to study the congealing tendencies of starch products precooked by procedures identical to those used in industrial practice, such as prepared adhesives and sizes.

Suggested improvements in the present type are better control of moisture evaporation from the sample during the test and better design in the mechanism by which the torque developed is transmitted to the recording device. When early models are used, the shaft of the measuring unit develops sufficient play, in time, so that viscous liquids produce feathery curves. It seems that a better way could be provided to control temperature than by surrounding the paste cup with a small air bath.

Several springs are supplied with the amylograph. By a proper choice of springs, a wide range in viscosities may be measured. Specific instructions are supplied by the maker for operating the instrument.

In application of the instrument to obtain a "paste history" of starch a slurry is made up of 35 g. of dry starch and 450 cc. of water. With a lighter spring adjustment this concentration is suitable for the accurate evaluation of unmodified starches such as corn starch. This concentration may also be used for a comparison of the pasting properties of modified starches, such as acid-modified corn starches of 20 and 40 fluidity, with unmodified starch. However, for the more accurate evaluation of modified starches and comparison with other modifications, a starch ratio of 55 g. of starch to 450 cc. of water is suggested.

The cup containing the starch slurry is set in motion, and the electric heating unit is turned on. While the temperature of the starch is raised at a uniform rate, the curve drawn by the instrument is plotted in terms of time units against viscosity units. It is desirable therefore to calibrate the curve during the period of heating and cooling, in terms of temperature. This is done manually, by watching the thermometer in the paste. At every 5° C. change in temperature (or more frequently, if desired), a mark is made on the curve by quickly moving the tracing pen through a small arc, which movement causes the pen to make a mark as the spring draws it back into position.

After a temperature of 95° C. is reached, the control is thrown over to the constant temperature setting, and changes in viscosity are noted. Viscosities normally fall off slowly. If the period of heating at 95° C. is extended beyond 15 to 20 min., the results become complicated by losses of moisture.

At the end of the period of cooking, the heat is turned off, and increases in viscosity for an agitated paste are noted as the temperature gradually decreases.

4. Alkaline Fluidity. Although the use of this test for evaluation of a starch has fallen into disrepute in some laboratories for considerations already given, a description of the method will be given for two reasons: First because the well known acid-modified starches were originally graded according to a fluidity scale by this method, and these ranges of modification by acid are still comparable to those used heretofore; secondly, the test is still employed by some industrial laboratories for controlling the manufacture of certain modified starches. In the latter case, when the complete history of a modified starch is known, the use of the test for this purpose is perfectly justifiable.

Five g. of starch, dry weight basis, are wet with 10 cc. of distilled water in a Pyrex beaker. At 25° C., 90 cc. of a 1% solution of sodium hydroxide are added, with stirring, and the stirring is continued for 3 min. from the time the sodium hydroxide is poured in. The mixture is allowed to stand 27 min. more at 25° C. At this time the contents are poured into a standardized glass funnel with a special tip and the quantity of starch solution which runs from the funnel in 70 sec. at 25° C. is noted. This amount, measured in cubic centimeters, is taken as the fluidity; *e.g.*, the acid-modified, thin boiling starches of commerce with a fluidity of 40 will be fluid enough for 40 cc. of solution to pass the funnel tip in 70 sec., with a fluidity of 60, 60 cc., etc. If 100 cc. of water are poured into the standard funnel at 25° C., 100 cc. will be delivered in 70 sec. Relatively then, water might be said to have a fluidity of 100 by this test.

Alkaline solutions of unmodified corn starch will hardly run through the tip of the funnel. Their fluidity is of the order of 1. In some laboratories where the alkaline fluidity of the thicker starches is compared it is customary to reduce the amount of starch used in the test to 2 g. or less.

When alkaline fluidities are no longer used to appraise the fluidity of modified starches, Kerr has proposed (6) that the fluidity may be indirectly determined by use of the Scott viscosity value, the determination of which has been given above, in the following equation.

$$\text{Fluidity} = \frac{2000}{\text{Scott test value}}$$

This particular Scott test is run with 28.35 g. of starch to 280 cc. of water. For example, if the Scott test of a thin boiling starch is 50 then it is a starch with a fluidity of 40.

The use of alkaline gelatinization of starch to prepare a paste for viscosity measurement has been practiced for many years by starch chemists. It was not until 1912, however, that the method was introduced to the corn starch industry by Buel (15) in substantially the form outlined above.

Certain oxidized starches (*e.g.*, the hypochlorite-treated corn starches of commerce), if free from buffers, give results by the alkaline fluidity test which are not related to the Scott test according to the equation above. The determined fluidity is much higher (viscosity lower) than that estimated from the Scott test. Indeed so characteristic is this discrepancy that it may be used as an aid in the

identification of an oxidized starch. Supplementary chemical tests may then be used to establish the type of oxidant used in the manufacture of the unknown sample.

5. Gel Properties.

A. Gel Strength—The gel strength of starch pastes has been measured by a variety of instruments embodying different principles. As might be expected, different characteristics in a gel may be measured by these tests, although the strength of the gel may or may not, depending on the view-point. Several of the instruments employed in practice are the rigidometer, penetrometer, Tarr-Baker jelly tester, and similar instruments. The rigidometer measures the rigidity of a gel, or its lack of elasticity, the Tarr-Baker (and instruments of that type) gives a value which is proportional to the force necessary to rupture the gel and which is also probably a function of the elastic limit. The results of tests with the blunt plunger type penetrometer are a combination of plastic and elastic effects. The tube type of plunger measures the resistance of the gel to a cutting action. The values so obtained need not necessarily show a correlation. Certain types of rubbery gels show high elasticity but fracture at values less than those required to break the structure of short, firm gels. Plastic gels such as those exemplified by modified tuber starches possess a low elastic limit, yet give a high reading by some of the penetrometer type of instruments. All of these varied characteristics are of importance, however, in considering specific industrial applications of starches.

B. Tarr-Baker Jelly Tester—Gel strength is conveniently determined by the Tarr-Baker jelly tester. This instrument was described by Baker (16) and modified by Tarr (17). In principle, a pressure is gradually applied to a plunger, of known area, resting upon the surface of the gel. The pressure is read at the instant that the gel breaks.

The directions are as follows: Weigh carefully 8 g. of unmodified corn starch or 12 g. of acid-modified, thin boiling corn starch, and other starches in proportion to their relative viscosity. Transfer to a porcelain cup, insert a stirring rod, and weigh. Add 100 g. of water. Cook in a boiling water bath for exactly 30 min., timing from the instant the sample is put in the bath. Stir the sample during the first 5 min., and then cover with a rubber stopper through which a hole is bored for the stirring rod. Stir for a few seconds at the end of 10, 20, and 30 min. Cool quickly with occasional stirring and add sufficient water to compensate for moisture evaporation. After mixing in the added water, if the latter is necessary, fill two aluminum moisture dishes, 55 mm. in diameter by 17 mm. in height. Cover at once with a film of light mineral oil. Place in a water bath at 20° C. for 1 hr.

Drain off the oil and place the dish under the plunger of the tester. With 500 cc. of water in the Tarr-Baker jelly testing jar at the start, adjust the flow of water so that the manometer column rises 60 cm. per minute. Read the manometer when the starch jelly suddenly breaks. Tests are made in triplicate and averaged. The gel strength is reported as the height (in centimeters)

reached by the liquid in the manometer at the breaking point of the gel, for a given concentration of starch to water in the paste.

C. Saare Disc Method—Among the first tests proposed for the evaluation of a cold paste was that of Saare and Martens (18), in which the weight required to withdraw a disc from a gel in which it was imbedded was determined. A modification of the method has been used by the author for several years. Details of the modified method follow: Starch is cooked in the same manner as described for the Scott test, a higher ratio of starch to water than in the latter test being used. For unmodified corn starches, 22.4 g. are used per 280 cc. of water. For modified starches between 30 and 50 g. of starch may be used, depending on the extent of modification. Immediately after the 15 min. cooking period, the starch is poured into a glass vessel, and the latter is filled to a definite mark. A circular metal disc of known diameter is suspended in the paste by means of a metal rod connected to the center of the upper surface of the disc. The metal rod is crooked at the top and is hung over a bar which rests on the top edges of the vessel holding the pastes. The length of the rod is such that, when suspended over the horizontal bar, it permits immersion in the paste of the test disc to a depth of 3 cm. A thin film of light oil is placed on top of the paste as soon as the disc has been inserted and properly centered. The test vessel and contents are now placed in a constant temperature bath at 25° C. and held in the bath for 24 hrs. At the end of this time the test vessel is removed and placed on a bridge over one pan of a large, but sensitive, beam balance. A specially made hook suspended from the beam of the balance just engages the crook in the rod attached to the disc. The temporary bar support for the suspended disc is of course removed first. Small size shot are added at a fixed rate to the other pan. The weight of shot is noted at the time when the disc fractures the gel. Fig. 48 shows an assembly for the determination of gel strength.

Some types of starch gels show a considerable deformation before a fracture actually occurs, as intimated above. One should not be misled into mistaking such deformations for an end-point in the test under discussion. An actual visible fracture is taken as the end-point.

The weight of shot measured, less the weight of disc and all connections to



FIG. 48. Apparatus for starch gel strength by modified Saare method. Photograph by H. Meisel.

the beam of the balance, divided by the exact area of the lower surface of the disc (about 3 sq. cm.) is taken as the gel strength. The result is expressed in grams per square centimeter for a given concentration of starch.

One shortcoming in equipment so elementary in design is that no guides are provided to maintain the rod attached to the surface of the disc in a perfectly vertical position. Some skill and practice is therefore required in adjusting this rod to a vertical position and maintaining it so during the period of test. Otherwise some slippage of the disc may result as the disc is pulled from the gel.

The method is, none the less, adapted to routine testing in practice and has a precision of 2 to 3%. This precision is more than ample for industrial practice, as can be appreciated from the fact that industrial grades of thin boiling starches vary about 20% in gel strength when one fluidity, *e.g.* a starch of 20 fluidity, is compared with the next, for instance a starch of 40 fluidity. The accuracy of

this method has been found greater by the writer than the Tarr-Baker or any other method of evaluating limits of strength in a gel. With such simple equipment many tests may be set up simultaneously by securing identically made discs and calibrated vessels to hold the pastes for testing. As for most other tests for paste evaluation, the major part of the experimental error involved is in the preparation of the paste, or gel, to be tested.

D. Penetrometers—The use of various penetrometers has been proposed, but most of these are not sufficiently sensitive to distinguish even between the various grades of acid-modified industrial starches. The writer's experience with the Bloom gelometer used extensively in the control of glue and gelatin manufacturing gives approximately the same order of values for starches with fluidities of 20, 40, and 60, all conditions of test being equal. Alexander (19) gives a discussion of this instrument and its use.

One type of penetrometer is, however, as sensitive and as accurate as the modified Saare method of determining gel strength. The paste is cooked as in the Scott test, between 20 and 35 g. per 280 cc. of water being used, depending on the modification of the starch. After being cooked, it is immediately placed in a closed, wide mouth container for storage in a constant temperature bath.

At the end of the aging period the cover of the vessel is removed and about a $\frac{1}{4}$ in. ls of the gel is cut off and discarded. The plunger of the instrument is now adjusted so that it just rests on the top surface of the prepared gel. A weight is imposed on the plunger in a receptacle on top of a rod, which is held

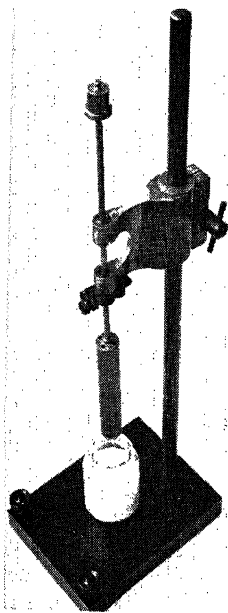


FIG. 49. Fuchs gel tester for starch pastes. (Courtesy of F. Fuchs; photograph by H. Meisel.)

in a true, vertical position by several guides. The bottom of the rod is attached to the top of the plunger. The latter consists of a sharpened, hollow metal tube that is highly polished. This tube is similar in appearance to a large cork borer. The plunger is released, and the time, in seconds, required for the hollow tube plunger to cut into the gel up to a certain depth is noted. This depth is determined by three markings on the rod or shaft affixed to the plunger. One of the guides is movable and is used as a point of reference. It is set at the lowest mark on the shaft just before the plunger is released. The time is noted when the middle mark passes and when the upper mark on the shaft reaches the point of reference. An instrument of this type has been constructed and used by the authors' associate, F. W. Fuchs (Fig. 49).

E. Rigidimeters—A very satisfactory instrument for determining the elasticity or the rigidity of a gel has been described by Brimhall and Hixon (20). The method of employing the rigidometer with starch pastes has been fully described by these authors.

The essentials of the instrument are as follows: A glass tube is suspended by means of a torsion wire into a column of paste, and the latter is allowed to gel and age at a fixed temperature. The torsion wire is suspended from a circular scale, quite similar to the arrangement in the MacMichael viscosimeter. At the end of the aging period the cylinder holding the column of paste is placed in the stand of the instrument which holds it in a fixed position. By means of a dial knob attached to the face of the circular dial, a small twist is developed in the wire. The angle of twist is noted. The corresponding angle through which the tube which is embedded in the starch gel moves is determined by the angular deflection of a beam of light which falls on a mirror attached to the glass tube. Determinations are repeated for different degrees of twist of the torsion wire. The originators of the method have given the method for determining the rigidity from the values observed. The angular degrees of twist, δ , applied to the wire are plotted against the corresponding deflections, w , in angular degrees. The slope of this curve δ/w is substituted in the following equation,

$$\frac{\delta}{w} = \frac{1}{R_1^2}$$

where E is the modulus of rigidity in dynes per square centimeter, N the torsional moment of the wire used, h the height of the paste on the inner cylinder, and R_0 and R_1 the radii of the inner tube and outer cylinder respectively. The wire used is standardized by measuring the oscillation time of a suspended disc of known mass. Then $N = 4\pi^2 MR^2/2T^2$ where T is the period of oscillation, M is the mass of the disc, and R is its radius.

In applying a torque to the wire, one should not turn through an angle so great that the structure of the gel will be in any way permanently injured.

6. Plasticity in Pastes. Several attempts have been made to measure the plasticity of starch pastes. In general, these have been based on the concepts outlined by Bingham (21). The rate of shear is plotted against shearing stress,

and the curve is extrapolated to zero rate of shear in order to obtain the "yield shear value." Hypothetically, this value is the force applied in overcoming plasticity in a fluid before the paste begins to flow. Application of the method to starch, however, presents certain difficulties. For example, Porst and Moskowitz (22) find that the curve obtained by plotting the rate of flow of a starch paste in a Bingham-Greene type plastometer against the applied pressure is not a perfectly straight line. It is difficult, therefore, to extrapolate these curves to zero rate of flow. Furthermore, it has been found by Farrow, Lowe, and Neal (23) and by Hatschek (24) that flow actually takes place below the yield value estimated, and thus the latter does not agree with Bingham's definition and has no physical meaning. It has also been observed that the results obtained with a starch paste depend to a large extent on the size of the capillary through which the starch is forced to flow.

The results plotted on a logarithmic scale give a more nearly linear line which can be extrapolated with greater ease. Farrow, Lowe, and Neal (23) therefore propose (and Rabinowitsch (25) gives a mathematical justification for the procedure) to plot the logarithm of the stress applied to the paste ($RPg/2L$) against the logarithm of the rate of shear produced ($4v/\pi R^3T$). If the slope of this line is denoted as M , and C is the point of interception, then the mathematical equation becomes

$$\log C = \log 4v/\pi R^3T - M \log RPg/2L$$

Not only do the results, so expressed, give a fairly straight line, but the position of the line is independent of capillary size. Moreover, it would appear that with suitable methods other types of viscosimeters may be used and the results, calculated in terms of shear and stress, may be correlated and expressed by the same constants as determined with capillary viscosimeters.

Brimhall and Hixon (26) have recently applied the above equation to a study of the flow characteristics of pastes made from a variety of starches and have shown that the equation holds for the results obtained by use of a specially designed viscosimeter of the capillary type. Their method and equipment would seem to be ideally suited for the measurement of flow in starch pastes.

Pastes at 5% concentration are cooked in a Pyrex test-tube suspended in a hot water heating bath. The test-tube is provided with a stopper through which is inserted a stirrer or, at the end of the cooking period, a capillary pipette. A 40 min. heating period at 90° C. is recommended. The paste is then cooled to 25° C., and the capillary pipette, with a capillary of known radius in centimeters (R) and known length (L) is introduced. The pipette is connected to a 3-way stop-cock, so that suction may be applied to fill the pipette to a given volume (V). The time of flow in seconds (T) is measured for various applied pressures, obtained from an air pressure line with a relief-regulating valve, which are read on a water manometer as centimeter head of water (P); g is the acceleration of gravity.

Capillaries should be between 0.035 and 0.075 cm. in radius, so that the time of flow is kept in the range between 30 and 200 sec. For unmodified or very

viscous starches, the weight of starch used should be adjusted so that comparable rates of flow are obtained. The cooking and cooling procedure should be standardized and the technique adopted rigidly followed.

7. Paste Shortness. This characteristic is one of the most commonly discussed properties of paste in industrial applications; yet the property is rarely evaluated except by casual inspection. As the product is being cooked or otherwise prepared, a paddle, ladle, or rod may be immersed in the paste and withdrawn to note whether the flow therefrom is stringy or short (which is the opposite property) in character.

If the restricted flow of such pastes is observed by the aid of a stroboscope, as for example the dropwise delivery from a small orifice, the shape of such drops as they emerge will be seen to vary progressively as pastes with progressively greater shortness are tested. Native corn starch pastes are comparatively short, whereas tapioca or potato starch pastes are normally stringy. Proper mixtures of the two types may be made to obtain pastes with intermediate properties.

The above observation may be made the basis of a test for shortness. The author has used and prefers, however, a more simply executed test, a description of which follows: Depending on whether shortness is to be estimated on the hot paste or cold paste, a weight of starch is mixed with that amount of water which, after the cooking in procedures otherwise comparable to a particular industrial application and at the temperature selected, will deliver 50 cc. from the orifice of a Scott viscosity cup filled with 200 cc. of the paste in 100 ± 1 sec. When these preliminary conditions have been fulfilled, the test is repeated, with the lowermost point of the Scott cup orifice about 2 cm. above the graduated cylinder used to receive the paste. The orifice is opened, and the eye is trained to the space between the orifice and cylinder. At first, the delivery of the paste will be observed to be a continuous thread. After a period, the delivery appears to be discontinuous, in part. Eventually, a point is reached at which the outflow changes quite definitely to a dropwise delivery. At that instant, the number of cubic centimeters of paste collected in the graduate is noted. This value may be taken as a relative measure of shortness; the lower the value, the shorter is the paste. Certain starches, modified to increase the short property of their pastes, will give values of 15 to 20. Native corn starch exhibits a hot paste shortness of 50. Unmodified tapioca will give a value of 125 under the same conditions of testing. The method was developed empirically after comparative observations on the hot paste viscosity of various starches by the Scott test.

8. Syneresis or Water Retention in Starch Pastes. Many pastes after aging will show a tendency to "bleed" or separate a liquid phase. This is particularly true of short gels with comparatively low concentrations of dry starch solids. This property is rather difficult to evaluate, especially in cases in which sufficient starch has been added so that a firm gel results. An evaluation may be obtained directly in some cases and indirectly in others by preparing a paste at such a concentration that it will remain fluid when cooled and aged. Two methods are suggested, each of which depends on the speed of filtration of the liquid phase.

In the first, a suspension of a selected clay is made up by mixing 30 g. of clay with 75 cc. of water. After a thorough mixing of the clay, sufficient starch paste is added so that the ratio of starch solids to clay is 15 : 100. Hence, 4.5 g. of starch are added. The total water present is now adjusted to 175 cc., and the mixture is stirred in a covered, high speed mixer for 10 min., whereupon it is transferred to a funnel fitted with a standard filter paper. The number of cubic centimeters collected in a given time interval is taken as a measure of the relative water retention of the starch. Obviously, the clay and filter paper used should be standardized as far as possible. A good grade of English clay should be selected, and a filter paper such as Munktell's No. 3 should be used.

For the second type of test, a stock supply of light weight, bleached but unsized paper stock should be procured. This is cut into squares measuring about 3 cm. on each edge. Just before the test is to be performed, one face of the paper (the wire face) is dusted with an indicator which changes color in the presence of moisture. For example, a powdered, intimate mixture consisting of 45 parts of cane sugar, 5 parts of soluble starch, and 1 part of du Pont N. E. methyl violet may be used as an indicator. The paste to be tested is thoroughly stirred up, and about 25 cc. are placed in a small porcelain dish. The prepared paper is placed on the surface of the paste with the dusted side up. With the aid of a reading glass, the time in seconds is noted for the indicator to change color. This value may be taken as a relative index of the water retention of the starch when a paste has been formed.

The starch is gelatinized in the manner recommended for preparing a paste for the Scott test. 10 g. of starch are used per 280 cc. of water. After the mixture is cooked, the covered beaker containing the paste is transferred to a bath at 25° C. At the end of 3 hrs. the paste is removed for test. About ten observations on a paste should be made and the results averaged. The results are expressed as seconds of water retention for a given concentration of starch. Water retention tests may and should also be determined at different starch concentrations. A more complete picture of the measured characteristic is given by the slope of the curve obtained by plotting water retention against starch concentration in the paste. Variables which require standardization are the paper and indicator used and a definition of the end-point with the indicator.

Water retention of pregelatinized starches, such as the roll-dried corn starch products of commerce, are very readily determined as follows: 5 g. of product, measured on a dry solids basis, are wet with a small amount of distilled water to form a dough. More water is added with stirring to form a suspension which is quantitatively transferred to a 100 cc. graduated cylinder. Sufficient water is now added to make the total volume up to the 100 cc. mark. After a complete mixing of the contents, the cylinder is allowed to stand at rest for 24 hrs. at 25° C. At the end of this time the line of demarcation is noted where the starch product appeared to have settled out from the water phase. The apparent number of cubic centimeters of the insoluble phase divided by 5 is taken as an index of the water retention of the product, per gram.

9. Gelatinization Temperature of Starch. Many methods have been proposed for the measurement of this important paste characteristic of a starch. Possibly the reason that divergent values are found in the literature for a given starch is the poor definition of the term. Naturally the choice of method for making the determination will, in a measure, depend on one's conception of the gelatinization point. For example, in gelatinization the granules first swell and finally collapse or tend to disintegrate if the heating is extended. If a technique is employed which takes advantage of the fact that the granules swell, such as by observing increases in the viscosity of heated suspensions of starch, it will be observed that the viscosity increases over a broad range of temperature. It is a matter of judgment, therefore, to locate the central portion of the range or the portion where the viscosity is increasing at a maximum rate. Simultaneous examination of these pastes, by means of a microscope, moreover, reveals several additional complications. Swelling of granules begins before the effect of increased viscosity becomes apparent by the use of many viscosimeters employed in practice. Moreover, in some cases, the viscosity continues to increase apparently after the granules have reached their point of maximum distention. Finally, not all the granules in many types of starch swell in exactly the same temperature range. In general the larger, more fragile granules swell at a lower temperature than the smaller granules of the same type of starch. In varieties in which there is a wide range of granule size and the sample is composed chiefly of the smallest and largest members, with few granules of intermediate size, a double gelatinization point may be observed by several methods.

Another characteristic of swelling granules is that they lose their activity in polarized light. The crosses commonly observed in native starch granules gradually become less distinct as a water suspension of the starch is heated. Finally they disappear altogether. Indefinite end-points are, therefore, the usual result of the use of methods based on this property, for much the same reasons as given above (28). What is more confusing, however, is that loss of birefringence cannot always be correlated with the change in viscosity. Many corn starch samples will have lost birefringence in all granules, 10° or more under the temperature at which significant increases in viscosity start to become apparent in some continuous viscosimeters (29).

Another characteristic of starch suspensions in water, heated to their gelatinization point is that the suspension becomes less opaque. This characteristic has also been made the basis for methods to determine the gelatinization temperature of starch. Some starches, however, give much more translucent solutions as they gelatinize than others. This consideration must be kept in mind in outlining methods for comparing various starches by their light transmission at various degrees of gelatinization.

The hydrogen ion concentration is a variable which should be controlled in all methods, since the temperature at which any starch gelatinizes is a function of the *pH*. Gelatinization temperatures reported in the older literature, especially for corn starch, are practically worthless inasmuch as the *pH* was not controlled.

Most corn starch, several decades ago, was made by an alkaline process and, as a rule, was finished at hydrogen ion concentrations higher than those now considered normal for corn starch. The presence of other specific ions or molecules should also be considered. The degree of gelatinization, other conditions being constant, is affected by the rate of stirring the sample, to a lesser extent by the rate of temperature increase, and, in extreme cases, by the ratio of water to starch present in the test samples. Samec (30) has reviewed some of the earlier methods used to determine the gelatinization temperature and has discussed at length the control of factors which alter the gelatinization temperature of a starch.

More recently, Morgan (31) has elaborated on a method based on the change in light transmission of starch slurries during the period of paste formation by heat. A photoelectric method is used to measure the change from cloudiness to translucency which occurs when a great variety of starches is heated with water. These include native white potato, wheat, tapioca, rice, corn, sweet potato, sago, and Florida arrowroot starches.

About 0.33 g. of a starch sample is rubbed up in 65 cc. of water and poured into a large test-tube. A small stirrer is set in the slurry and turned on. The tube and contents are immersed in a glycerol bath which gradually raises the temperature of the starch 2.5°C. per minute. Light from a 100 watt projection bulb passes through a collimating lens, on through the tube in which the paste is formed, and onto the photoelectric cell. The intensity of the light source is maintained by a constant voltage regulator. The illumination is initially adjusted, just before a determination, by use of a slide-wire resistor, to a standard value of 250 microamperes or 500 foot candles. A microammeter is attached to the photocell. The values for light transmitted, in microamperes, are plotted against the temperature ($^{\circ}\text{C.}$). Characteristic curves are obtained from each starch. Modified starches such as the so called oxidized starches and acid-modified starches of commerce show progressive alterations in the curve.

Wheat starch shows a double gelatinization range referred to above. Sago and Florida arrowroot show an initial increase in opacity which is ascribed to a disintegration of aggregates into individual grains. It is claimed that the start of paste formation indicated in this method by an abrupt rise in the transmission corresponds to the first appearances of paste formation as shown by a decrease in the number of granules showing birefringence. This would seem to be confirmed by comparing the data of Morgan on corn starch with that given by Schoch (29). Although Morgan directs attention to the temperature at which paste formation begins, the curves can very readily be used for an estimation of the half wave height, which might be taken as the gelatinization point by this method. Wheat and starches behaving similarly would have two gelatinization points.

However, when these curves are compared with those obtained by plotting viscosities against temperature, *e.g.* replotting Brabender amylographs on rectangular coordinate paper, and estimating the half wave height of these

curves, a discrepancy in the result obtained by the two methods is disclosed. For example, by the Morgan method the gelatinization point of corn starch appears to be 77° C., whereas by the Brabender method a value of 85° C. is obtained. Hence gelatinization point is mainly a matter of definition. From an analysis of the supplementary data available (29, 31) it would appear that the first method is the more reliable. Using the Brabender amylograph for the purpose described, one should take as the gelatinization point that temperature at which the graph first shows a decided change in slope upwards. For corn starch, unmodified, at normal pH this would be 78° C.

To show the extreme variation in the calculated results obtained with the different methods employed to determine the gelatinization point of starches, reference is made to the recent work of Mullen and Pacsu (32). In presenting a review of the general subject of gelatinization, these authors used an instrument of the type described by Caesar (12) to measure the increase in the consistency of 15% starch pastes, and they employed a temperature increase of 3° C. per minute. When the increase in the wattage input to the stirring motor is plotted against the temperature, very sharply rising curves are obtained in the region of gelatinization. The half wave temperatures found are as much below those obtained from results on light transmission as the half wave temperatures of the Brabender amylographs are above. For example, for corn starch the gelatinization temperature (defined by the authors as the temperature at which the rate of increase in consistency reaches its greatest value) is given as 68.5° C. in pure water. The results for other starches are comparatively low. Furthermore, the authors note that for the starches studied, potato, tapioca, wheat, corn, and rice, the gelatinization temperature in water is inversely related to the average granule size of the sample. According to this method it would seem that the probable gelatinization point is more nearly the temperature at which the starch exhibits its maximum consistency.

Although the technique of Morgan is recommended for more careful studies, the author has obtained comparable results with a very simple and quickly performed procedure used for many years. In routine work the experimental error is about 1° C. for an experienced operator.

Starch slurries are made up at 10% concentration for unmodified corn starch and at concentrations for other starches depending on their relative hot paste viscosity as compared to that for corn starch. For example, for the average, acid-modified thin boiling corn starch, 15% concentration is recommended. Unless results are reported to the contrary, the pH of the slurry is adjusted so that the final starch has a pH of 5.0 to 5.5. The slurry is placed in a metal cup provided with a stirrer and thermometer. The cup is then placed in a water bath having a temperature about 5° C. under the anticipated gelatinization point. The stirrer and heating unit are turned on gradually. When the temperature of the slurry is about 10° below the anticipated gelatinization point, the rise in temperature is regulated at 1° C. per minute. Mechanical stirring is discontinued and the stirring continued with a glass rod, by hand. The temper-

ature at which the first thickening effect of the slurry is discerned and also at which the paste has become so thick that further increases cannot be noted by inspection is recorded. For most starches this range is about 4–6°. The average of the two temperatures is taken as the gelatinization point. For unmodified corn starch at pH 5.5 this value is 77° C.

10. Protective Colloid Effect of Starch Sols. Specific tests of this nature are difficult to outline, inasmuch as the effectiveness of the starch will depend on the properties of the material to be suspended, the nature of the suspensoid to be precipitated, whichever the case may be, or on the properties of the non-miscible liquid to be emulsified. Also, it is possible that additional substances which may be present may affect the suspending properties of the starch product. It would seem more sensible to standardize, as tests, small scale operations comparable to the exact use to which the product is to be put.

Starches are used as suspending agents, and one of the chief uses of this type is to suspend clays in preparing heavy sizes or coatings. An illustrative test given below is based on this use. Starch products are used as floatation agents in certain mining operations and as precipitants in others. In the latter instance, the sedimentation of solids from the wash water from coal may be noted. Starches are used as emulsifying agents, particularly in such food products as salad dressings. Starches are claimed to be foaming agents, particularly by those who, because of poor control in the preparation of adhesives or sizes from starch, are perpetually troubled with foaming products. In such cases investigated by the writer, however, conditions of foam could always be traced to some extraneous material such as might be present in the water supply at the point of use, unanticipated high pH indicative of free alkali, or even accidental impurities in the starch product or to reagents or additives which should not have been used. A high content of soluble proteins in enzymes of weak diastatic power is an example of the latter. Moreover, in instances in which the author has deliberately attempted to make a marketable foaming agent from starch, these experiments have met with utter failure. In general, it may be said that starch is not a foaming agent and is of such weak emulsifying power that rather large concentrations of starch sol are required to hold up such fats as the vegetable oils (33, 34). Oxidized starches are among the best types of starch for protecting suspensoids; unmodified corn starch, particularly if it is partly gelatinized and then dried in a manufacturing process, is a good sedimentation agent for many suspensoids.

The clay-suspending ability of a starch may be conveniently determined as follows: 1 g. of the starch product, dry weight basis, is thoroughly gelatinized in about 75 cc. of water by a standard cooking procedure and cooled. An excess of English clay is now stirred into the starch, for example 2 to 3 g. After thorough mixing, the mixture is quantitatively transferred to a standard 100 cc. graduate with sufficient water to make a total volume of 100 cc. After a second thorough mixing of the contents, the graduate is stoppered and allowed to stand 20 hrs. at 25° C. The topmost 80 cc. are now withdrawn, and a determination of the

dry solids is made by evaporation on sand and then by heating for 4 hrs. at 120° C. in a vacuum oven. The weight obtained divided by 0.8 and minus 1.0 is taken for comparative purposes as the relative clay-suspending ability of the starch at the pH noted for the suspension.

Alkalies in general, such as sodium carbonate, borax, and tetrasodium pyrophosphate, are fluxing agents for clay. Obviously, therefore, the pH is a variable in the above determination which should be controlled. The clay used for testing should be standardized, as some clays are more difficult to suspend than others.

11. Supplementary Tests on Paste. Quite frequently supplementary tests are performed which supply additional information of practical importance. Many of these are extensions or combinations of the types of tests already given. For example, it frequently becomes important to know the comparative rate of gel formation in a starch paste at a given temperature. Otherwise, on the basis of a preliminary examination, a starch might be incorrectly applied. In such cases, a series of duplicate tests for gel strength may be set up, the same starch being used in each case. At stated time intervals a sample is removed from the constant temperature bath and an observation is made. Gel strengths are then plotted against the time measured after the cooking and cooling operations. A curious effect may be observed with the procedure outlined. The presence of very small amounts of fats increases the rate of gel formation in corn starch. After a short period of time, such a starch will show a higher gel strength (and cold paste body) than a defatted corn starch. However, after longer periods of time the defatted starch will surpass the other starch in gel strength (and cold paste body).

One of the most important characteristics of a starch for many industrial uses is its change in properties under the influence of mechanical stresses such as pressure, shearing action, and so forth. In many mills employing starch products it would seem that very little thought had been devoted to the location of equipment for the preparation of the starch. Accordingly, paste kettles will be found in out of the way places, frequently in the basement or in other parts of the building unsuited for any other use. Equipment for application of the prepared adhesive or size may therefore be located at some distance from such paste kettles. Frequently, rather considerable pressures are required to pump these pastes to their point of use. It is important to know to what extent the viscosity of such a paste may be expected to be reduced after such treatment, whether its tendency to gel will be increased when the pump and pipe lines are left full of paste during a periodic shutdown of the plant as over week ends, and so forth.

Again, it may be observed that some portion of a moving part of the mechanism which applies a paste is of such poor design that undue shearing forces or cutting actions are set up in the starch supply. These tend to create a non-uniformity in the viscosity of the starch paste. For example, supply boxes at the machines are often provided with an overflow to larger supply tanks. In

such cases, the first part of a batch of adhesive or size may be of substantially higher paste body than the last of the batch.

No standard tests are suggested. It is recommended that equipment be set up to duplicate the particular physical stress observed which is to be compensated for by selection of a specially made starch product. Viscosity, or cold paste body, and other pertinent properties are observed before and after stated periods of treatment. Care should be taken that the pH and temperature correspond with those of the paste used in the practical application.

12. Color of Starch. The color of starch is conveniently measured by the use of the Brice photometer or a similar instrument. The percentage of light reflected from the smooth surface of the powdered starch sample is determined by means of a photoelectric cell. When the instrument is balanced for a standard white surface, usually one prepared from MgO, which is assumed to give 100% reflectance, the percentage reflectance of the starch may be read directly. It is preferable to make two determinations, one with a green filter in the path of the light source and the other with a red filter. Both filters are supplied with the instrument. The percentage deviation from white may then be expressed as

$$100 - \frac{\% \text{ reflectance by green light}}{\% \text{ reflectance by red light}} \times 100$$

In the determination of color by this method, errors introduced such as by the brilliance of the starch sample are minimized.

The visual inspection methods depend on the use of standard samples. Other starches are then graded by direct comparison with these standards. There are three general methods which require very little practice to enable one to match an unknown with these standards. The first two require that the starch be dry and powdered, preferably to a given range of particle size, as for example that passing through 200 mesh copper sieves and retained on 300 mesh.

In the first, a small amount of sample (about 0.5 g.) is placed in the center of a jet black sheet of plate glass. Small samples of standards are now placed in a circular arrangement around the unknown and at such distances that when a sheet of clear plate glass is gently pressed on all starch samples the surface of the starches will be continuous, one with the next. This surface is examined by reflected daylight, free from direct sun rays or reflected glare. The unknown is said to match that standard in which no line of demarcation can be discerned between the surface of the unknown and the standard.

The second method is the so called "stab test." The standard samples are placed in uniformly shaped glass bottles of equivalent size. The unknown is placed in a similar bottle. Each bottle should contain the same volume of starch. They are placed on a dark colored stand in reflected sunlight, and a hole is formed in each sample of starch, in turn, with a glass rod which is forced gently down from the top of the bottle until it strikes the bottom. Care is exercised in removing the rod, lest the hole formed become materially larger or

smaller through a disturbance of the surrounding starch. The eye is now trained on the various holes made, and the color perceived in the unknown is matched against the standard which most nearly approaches it.

The third method involves cooking the starches in dilute suspension, *e.g.* 2 g. in 100 cc. of water, in glass equipment and by a standard procedure. When cooked, the standards and unknown are cooled to 25° C. and compared by transmitted and reflected light after a given interval of time. Observations by this method may be complicated by opacity which in turn depends on the type of starch, degree of gelatinization, and retrogradation effects. The color developed may also be influenced by a variation in the pH of the sample. Moreover, color comparisons by this method may not be correlated with those obtained on the dry samples. However, the former are considered more reliable when the starch is to be applied as a paste rather than used in dry, powdered form.

More recently a test has been proposed in an attempt to evaluate the color of a starch, in particular of corn starch, quantitatively on an independent scale. The method presupposes that all of the color of starch is imparted by carotenoids such as those present in unmodified corn starch. This, of course, is not necessarily so, and the test must therefore be used with certain reservations in mind.

Ten g. of starch, dry basis, are shaken in a glass-stoppered flask with 100 cc. of 70% alcohol by volume. The flask is then centrifuged and the color of the clear liquor determined in the Lovibond type colorimeter on the Lovibond scale, with 70% alcohol as the standard reference fluid.

13. Odor of Starch. The odor of corn starch products is noted by inspection. pH and moisture determinations are first made, since these are variables affecting the development of odor. A clean, 8 oz. glass-stoppered bottle is half filled with the sample which is then placed in a constant temperature oven at 60° C. It is gently shaken and inspected once a day, and the length of time in days during which the product remains stable at a given moisture and pH is recorded.

14. Mobility of Dry Starch. A test adapted to the equipment found in most laboratories is as follows: 200 g. of the powdered starch are placed in a 200 mesh copper sieve such as the standard type supplied by the W. S. Tyler Company. The circular sieve is supplied with a cover and a receptacle to catch the starch passing the sieve. The assembly is placed in a Rotap shaker and shaken for exactly 30 sec. The percentage weight of starch passing the sieve is taken as an index of its mobility. Starches considered as mobile will pass 85% or more of the 200 g. placed on the sieve. The pH and moisture content of the starch should be noted, since they may be variables affecting mobility.

Occasionally the test is carried out on a larger scale in the mill. For example, 100 lbs. of starch may be dumped on a standard sieve consisting of a 48 mesh wire gauze attached to an eccentric making 120 r.p.m. If the sieve is of such size that about a 6 in. layer or so is present on the screen when the bag of starch is dumped, an immobile starch may require as much as 30 min. for the starch to pass the sieve. A mobile starch will pass through the sieve in 2 to 5 min.

15. Miscellaneous Tests. Other miscellaneous tests are applied in industry for the evaluation of a starch for general or for a specific use. Many of these are described in the sections on reactions or uses. For example, a control is maintained for starches specially prepared for diastatic liquefaction by the user, and it is the common practice to evaluate all batches of starch produced by some standard method for their susceptibility to such liquefaction.

Naturally, industrial starches are analyzed for ash, protein, fat, pH, and other characteristics, and a close control is kept by microbiological tests. The usual chemical tests are based on the Methods of the Association of Agricultural Chemists which are revised and brought up to date periodically. Bacterial counts, such as for the various classes of thermophiles, are usually made in accordance with procedures approved by the food manufacturer or a group of them, as, for instance, the National Canners' Association.

Occasionally specifications allow only an infinitesimal minimum of some substance; e.g., iron. In most of these cases, there is little difficulty in finding a suitable method in some standard text on analytical chemistry.

Sair and Fetzer (35) have made a careful review of methods for the determination of moisture in starch. They find that either a toluene distillation or drying in a vacuum oven at 100° C. gives reliable results as to the true moisture content.

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SECTION III. PROPERTIES

In the outline of the physical and chemical properties of starch which follows, the primary purpose is to present and correlate information pertaining to the structure of the component molecules and to the organization of these molecules within the granule, so that the behavior of starch can be interpreted in terms of fundamental principles. In view of the recent progress that has been made in this field of carbohydrate chemistry, it is believed that the problems pertaining to it have been sufficiently clarified to enable the presentation of certain concepts which although admittedly incomplete, nevertheless, furnish satisfactory working hypotheses. It was further believed that the application of these hypotheses, particularly that the behavior of the starches depends not only on the properties of its diversely constituted components but also that its behavior is influenced by the mutual effect of these components on each other, will, to a large extent, eliminate the uncertainties and supplant the empiricism which has prevailed in starch chemistry and technology.

That starch is heterogeneous is no longer seriously questioned. Sufficient evidence has accumulated to prove that the common starches are composed of at least two structurally different types of molecules. To continue to treat the subject with the view that starch is a chemical entity or to ignore one constituent by assuming that it is a component of minor importance and that its presence may be regarded as of the nature of an impurity can only lead to a perpetuation of a chemical literature on starch replete with incongruities.

Although an attempt has been made to divide the subject matter for discussion into two chapters, physical and chemical properties, the nature of the substance discussed makes a sharp and complete division along these lines very nearly impossible. Some repetition in the discussion of several topics has therefore been a natural consequence.

CHAPTER VII

PHYSICAL PROPERTIES OF STARCH *

DEXTER FRENCH

1. Introduction. Within the past few years, new and improved methods of isolating starch components have induced a considerable renewal of interest in research on the structure of starch (1). The older starch "fractions" were

* Some of the material presented in this section is the result of published research carried on in cooperation with others at Iowa State College, Ames, Iowa. The author wishes to acknowl-

poorly characterized and often highly degraded from the natural state. Previously, there had been a considerable diversity of opinion on just what factor or factors were responsible for the differentiation of starch fractions; some believed starch could be separated into its components by making use of differences in solubility; others believed the relative presence or absence of phosphorus or other inorganic constituents (impurities, perhaps) influenced the properties of the basic starch substance.

In the main, these older views have been eliminated, and at the present time an attempt is being made to ascribe the properties of starch or any of its fractions to the basic starch chain or its ramifications. On the whole, two extremes of starch types are recognized: *amylose*, simple straight chain starch (analogous to cellulose in chemical structure), and *amylopectin*, in which the regularities of the fundamental starch chain are interrupted by frequent branching. The structure of glycogen (animal starch) is similar, but more highly ramified.

Clearly, attempts to investigate the fundamental behavior of starch in any chemical or physical system should be made on the homogeneous constituents rather than the whole starch. However, for lack of homogeneous starch preparations, much of the older literature was confused and even contradictory. The availability of chemically homogeneous starch components for physical examination not only facilitates research, but also renders the results reported less open to question. Although much painstaking research on the physical properties of starch has been carried out, much of this must be repeated on the homogeneous starch fractions before it can have any fundamental significance. We are therefore just beginning to build up a reliable body of literature on the physical behavior of starch substance.

2. Physical Properties of Native Starch Granules. Under the polarizing microscope, starch granules appear as somewhat distorted spherocrystals (2). The typical birefringence may be accounted for either on the basis of an actual crystal structure or on the basis of some other orderly radial or tangential distribution of starch chains. Frey-Wyssling (3) has measured the birefringence in some starch granules; she points out that the positive sign of birefringence (i.e., with respect to the radius of the spherule) indicates a radial distribution of extended chains rather than coiled helices as suggested by Hanes (4) and Freudenberg (5). The magnitude of the birefringence (0.015) may be compared with that of cellulose (0.067). When leached with dilute sulfuric acid for several months, approximately 50% of the starch granule is dissolved (2). However the resulting modified starch granule is as highly birefringent as ever, indicating that very little, if any, of the crystalline material is destroyed by this treatment. Furthermore, the crystalline residue must have a birefringence roughly twice that of the average of the whole granule. The starch product resulting from such prolonged hydrolysis *in the granule* has been found to consist almost entirely of an unusually homogeneous crystallizable compound of low molecular weight called amylopectin. Amylopectin spherocrystals resemble starch granules closely, and the x-ray diagrams are indistinguishable from those of the best

undegraded starch preparations. Chemically, amylopectin is a chain of approximately 25 glucose residues, linked with typical starch unions, and containing very little or no branching. It may be inferred from these facts that the amylopectin molecules (or residue) make up the crystalline regions in starch; the crystallites are connected by heterogeneous regions of non-crystallinity which are more susceptible to the action of the hydrolyzing acid, while the organized crystallites resist hydrolysis. It does not follow that discontinuities in the starch molecules necessarily coincide with the crystallite boundaries, but rather that any one starch molecule may partake in several crystallites simultaneously, with any irregularities in the molecule lying in the amorphous regions.

The foregoing structure is supported by the physical behavior of starch granules on grinding or other types of granule mutilation such as strong desiccation. Microscopically, the effect can easily be observed by spreading a few starch granules on a slide and applying pressure to the cover-glass. The granules are seen to become distorted and lose their birefringence immediately; similarly, the x-ray diagram of physically mutilated granules becomes entirely amorphous in character. In other words, it is possible to "grind out" the crystallinity of starch without affecting its chemical properties significantly.¹

This behavior is in marked contrast to that of ordinary crystalline compounds, such as sodium chloride or sucrose, which maintain their crystalline structure through similar treatment. It indicates that application of physical stress to the granule causes a disarrangement of molecules with a resulting breakdown of crystallinity rather than merely breaking the crystals apart, for in no case does the bulk of the particles obtained on grinding approach the size of the ultimate crystallites.² This, in turn, indicates that the crystallites are held together by stronger than crystalline forces; *i.e.*, by main valence chains. Otherwise, the crystallites would merely be broken apart from one another to relieve the stress, with no loss of crystallinity.

The crystal structure of the starch granule bears little or no relationship to the source or type of starch. Starch granules from corn and waxy maize, which represent extremes of starch types, can be distinguished under the microscope only by means of the iodine reaction; furthermore, their x-ray patterns are only slightly different (6). In fact, starches from all sources, natural and synthetic, with the exception of glycogen, can be recrystallized to give crystal x-ray patterns which are identical with each other (7, 8).

3. Crystalline Forms of Starch.

A. A and B Configurations—Two main types of crystal structure have been found in native starch granules. These two extremes have been designated "A," represented by cereal starches, and "B," represented by the tuber starches, while types intermediate between the extremes have been called "C" types (7).

¹ Of course, if grinding is continued long, the molecules are reduced in size, and the reducing power increases noticeably.

² On the basis of the width of x-ray diffraction lines, the crystallites have been estimated to be 100 Å. in size. This is roughly one-fiftieth of the visual limit.

On crystallization of starch pastes from any source, A, B, or C types of crystallization can readily be obtained, depending on the temperature of preparation (8). In general, the B type of crystallization is obtained by evaporation of pastes at room temperature or by precipitation of insoluble starch by freezing or retrogradation, the C type at a temperature somewhat above room temperature, and the A type up to about 80–90° C. At higher temperatures amorphous preparations are usually obtained. The crystal unit cell parameters of the A and B types found by Bear and French are (6):

Type	$a_0(\text{\AA.})$	$b_0(\text{\AA.})$	$c_0(\text{\AA.})$	α	β	γ	$V(\text{cu. \AA.})$
"A"	15.4	8.87	6.18	87.0°	86.9°	92.8°	843
"B"	16.1	9.11	6.34	90.0°	90.0°	90.0°	930

Since the molecules are optically active, the only space group allowed for the triclinic unit cell is C_1 , a space group which is incapable of permitting crystal symmetry.³ The unit cells contain 4 glucose residues or two maltose groups, while the cellulose unit cells also contain 4 glucose residues or two cellobiose groups. However, cellulose crystallizes in the monoclinic system, and the crystal symmetry allows a 2-fold screw in the molecular chain (9). Presumably starch could also have a 2-fold or higher screw axis, but evidence for this is lacking at present.

A study of oriented amylose fibers and films by Rundle, Daasch, and French (10) partially confirms the crystal unit cell parameters of the B type configuration given by Bear and French. Two of the original dimensions appear to be correct but the dimension along the molecular axis is apparently 10.6 Å. Table VII gives a comparison of the unit cell dimensions of starch, maltose, cellulose,

TABLE VII

	$a_0(\text{\AA.})$	$b_0(\text{\AA.})$	$c_0(\text{\AA.})$	β
Starch	16.11	10.6	9.11	90°
Maltose (hydrate)	10.7	15.2	4.9	82.5°
Cellulose, native	8.35	10.3	7.95	84°
Cellobiose	11.1	13.2	5.00	90°

and cellobiose. The orientation of the molecules in maltose, cellulose, and cellobiose may be determined by the optical properties of the crystals or fibers and in each case the dimension closest to 10 Å. represents the length of the biose molecule or residue. The same conclusion applies to starch. However, the reason why the length of the primitive translation for starch should be greater than for cellulose is not readily apparent. Possibly the pyranose rings in starch are distorted, or possibly the α -glycoside linkage is sufficiently flexible to allow the formation of a longer unit cell.

B. V Type Configuration—When starch pastes are precipitated with alcohols or some other precipitating agents, an entirely different type of crystallization takes place (7). The x-ray diffraction pattern of alcohol-precipitated starch

³ Other than 1-fold axis, or identical symmetry operator, common to all crystals.

("V" pattern⁴) is much simpler than the A or B type of pattern, indicating a more symmetrical arrangement of molecules, and starch thus precipitated differs notably from granular starch in other respects (11). Freshly precipitated starch of the V type is readily dispersible, often in cold water; on standing the material gradually loses its solubility in water, and, if allowed to stand moist, retrogrades to the B configuration. A most striking singularity of alcohol-precipitated starch, *i.e.* starch of the V configuration, is its ability to take up large amounts of iodine vapor, even though quite dry (12).

Starch pastes precipitated with ethanol or propanol are coarse and formless, and the V pattern consists of but three or four more or less sharp lines. When butanol or amyl alcohol is used, a fine crystalline precipitate slowly forms (13), and the x-ray pattern is relatively rich. However, when the precipitate is dried, the simple V pattern is obtained. Starch precipitated with tertiary butyl alcohol gives an x-ray pattern similar in appearance to the ordinary simple V pattern, but the ring diameters are somewhat smaller, indicating a larger lattice. Starch precipitated with iodine and potassium iodide gives a fair V type of pattern (11).

The x-ray pattern of starch of the V type which has absorbed about 20% of its weight of iodine vapor is a simple appearing pattern bearing no relationship to the iodine crystal pattern but resembling strongly the V pattern. On calculation of the interplanar spacings, it is found that all strong diffractions can be accounted for on the basis of a simple hexagonal reciprocal lattice of two dimensions, which has a primitive translation of 13.0 Å. Such a lattice would be expected of closely packed cylinders 13 Å. in diameter (12). This same structure can be transferred to V starch.

There is every indication that in starch of the V type the molecule has a helical configuration in contrast to the extended molecular configuration of starch of the A or B type. Although a helical configuration has been postulated for starch, the fact that granular starch is in an extended configuration led some investigators to discount earlier evidence for a helical starch molecule. However, this earlier evidence was based on the behavior of starch in solution, and need not bear any relationship to the configuration of solid crystalline starch. Space-filling helical models of amylose molecules are approximately 13 Å. in diameter, in agreement with x-ray results.

C. Crystalline Amylose—Crystals of butanol-precipitated amylose are quite birefringent under the polarizing microscope, although not to the same extent as granular starch (14). Under certain conditions of crystallization, the crystals are more or less rectangular platelets which are pseudouniaxial and birefringent negative, the pseudooptic axis being normal to the platelet surface. On treatment with very dilute iodine solution, the crystals take up iodine readily and become extremely dichroic, indicating that the iodine molecules are highly oriented within the crystals. Since light having its electric vector normal to

⁴ *Verkleisterungsspektrum*, or paste pattern.

the platelet surface is extinguished while all other light passes practically without absorption, the iodine molecules must lie normal to the platelet surface.⁵ The only structure consistent with the foregoing properties is an arrangement of helical starch molecules oriented with the helical axis normal to the platelet surface. The iodine molecules taken up by the amylose lie on the helix axis and are held there rigidly oriented normal to the surface (15).

As it first comes down, the total butanol-precipitated fraction of starch is in the form of 6-sided rosettes which are probably aggregates of rectangular platelets. The rosettes have the same characteristic optical properties before and after treatment with iodine, and the x-ray diffraction patterns are identical.

D. Amylose Films—Hot solutions of starch, and especially of amylose, tend to form strong insoluble surface skins which can be removed and washed readily (13). These films are strongly birefringent, even when soaking wet, and are uniaxial negative with respect to the film surface normal. In converging polarized light these films exhibit a typical but weak uniaxial negative interference figure. The starch chains are evidently grouped parallel to the film surface, judging from the optical characteristics and the fact that the direction of most rapid increase in size of a growing crystal or crystalline aggregate is usually normal to the long axis of the molecule which, in the case of the film, is also normal to the film surface. From amylose films are obtained oriented x-ray diffraction patterns which may prove of value in the interpretation of the crystal structure of starch (12). Already the oriented patterns have shown that the primitive translation along the amylose chain is 10.6 Å., *i.e.*, the length of an anhydro maltose unit. Moreover, with oriented films it is possible to examine the degree of swelling on treatment with swelling agents in directions parallel and normal to the amylose chains. On treatment with dilute iodine solutions, the films become dark blue or black, but not noticeably dichroic.

4. Swelling, Gelatinization, and Retrogradation of Starch. The breakdown of the structure of the starch granule on heating in water takes place in three quite distinct phases. During the first phase, water is slowly and reversibly taken up, and limited swelling occurs. The viscosity of the suspension does not increase noticeably. The granule retains its characteristic appearance and birefringence, and upon cooling and drying, no obvious changes can be observed. Within a small range of temperature at approximately 65° C. (the exact temperature being a characteristic of the variety of starch) the second phase of swelling starts; the granule suddenly swells, increasing many times in size, taking up a great deal of moisture and rapidly losing its birefringence. The second phase of swelling is also marked by a rapid rise in the viscosity of the starch suspension, and upon cooling the granules are altered in appearance, most of them having lost their structure and birefringence. A small amount of the starch has become solubilized, as can be shown by treating the supernatant liquid or centrifugate with dilute iodine solution. During the third phase of swelling, which takes

⁵ Iodine molecules absorb only light having the electric vector parallel to the axis of the molecule.

place with increasing temperatures, the granules become almost formless sacs, and the more soluble part of the starch is leached out. Suspensions containing only a few per cent of starch are so filled with the swollen granule sacs that on cooling a rigid gel is formed.

The exact mechanism of swelling, the second phase of which may be said to be the active phase, is somewhat obscure in spite of much investigation and speculation. The swelling may be induced at room temperature by many chemical agents, notably alkali and metallic salts. By controlling the concentration of these agents, it is possible to reduce the speed with which the second phase of swelling takes place for convenience in microscopic examination.

By using swelling agents, one can observe the formation of a bubble in the interior of the granule during the second phase of swelling (16). This bubble grows in size until the granule becomes weakened and the bubble rapidly diminishes and usually entirely disappears. This phenomenon indicates that during the rapid swelling process the interior of the granule is a region of very low pressure, so much so that a void is formed and expands at the pressure of water within the system. The forces producing swelling must be largely in a direction tangent to the granule surface, since a radial expansion would not reduce the pressure in the interior of the granule.

Some insight into the nature of the swelling forces may be gained through the observation of the swelling of oriented films in which the molecules lie parallel to the film surface (12). On treatment with swelling agents, a film increases approximately 50% in the surface area, while the thickness increases to about 3 to 5 times its original value. The only interpretations of these facts which is consistent with the physical structure of the amylose film is that the amylose molecule swells in a direction normal to the axis of the molecule without any decrease in length. Since the amylose film is constrained by the molecular valence forces of the extremely long amylose chains, the bulk of the swelling must occur in a direction normal to the film surface. This direction is approximately normal to all the molecular chains, and there can be no constraint to the swelling. When swelling takes place normal to the starch molecules in the granule, it produces the observed tangential expansion and lowering of the interior pressure.

Starch granules which have been solubilized by the action of acids are no longer capable of swelling, but on treatment with hot water the granules disintegrate and pass into solution. The solubilization breaks the starch down to molecules of smaller size which are incapable of forming the giant networks characteristic of the swollen granules.

Dilute pastes consisting largely of swollen granules may be thinned to a large extent by boiling or other mechanical treatment, such as passing through a homogenizer. Such dilute pastes have less tendency to set rapidly to strong gels, but at higher concentrations gels are readily formed, even from thoroughly dispersed starch. These more concentrated gels depend for their rigidity on the starch molecules, especially the amylose molecules with straight

chains. In this connection it is significant that the highly branched glycogen cannot be induced to gelatinize or crystallize, while amylose forms gels in relatively dilute solutions.

Solutions of starch which have aged at room or lower temperature undergo the phenomenon of retrogradation. A part of the starch aggregates progressively, and finally forms an insoluble microcrystalline precipitate.⁶ The aggregation of starch makes it unavailable to the action of enzymes, even though the aggregates remain "dissolved;" *i.e.*, they do not precipitate out. The retrogradation process may be hastened by freezing aqueous solutions; in this way, ordinarily stable solutions may be forced to retrograde. Although some amylopectin preparations have a tendency to retrograde from solution, the property is greatly exaggerated in pure amylose solutions. Even 1% solutions of (corn) amylose will deposit practically the whole amount of solute in the form of crystalline sediment in the course of a few days.

The process of retrogradation takes place even in the solid state. The staling of bread is thought to be such a process, and starches precipitated by alcohol and left moist readily retrograde. Retrogradation is arrested by swelling agents, by keeping preparations above room temperature, or by removing the moisture.

5. Fractionation of Starch and Properties of Starch Fractions. The multiple amylose theory of starch composition rests in the main on efficient methods of fractionating starch into its ultimate components. Quite recently new and improved methods of fractionation have been developed which allow the isolation of undegraded starch components which are basically different in structure and have quite different physical properties. It has also been possible to synthesize *in vitro* types of homogeneous starch whose properties are practically identical with natural starch fractions. According to Meyer (1), *amylose* is used to designate unbranched starch chains, while *amylopectin* represents the component of starch that has branched chains. It must be noted that these definitions are on the basis of chemical structure, especially with respect to the presence or absence of branch linkages, while the older definitions were on the basis of such properties as solubility, enzyme digestibility, iodine color, etc. Although it is true that many other criteria may be used to distinguish between amylose and amylopectin, ultimately the burden of proof of structure of a starch fraction must fall on painstaking chemical analysis. Meanwhile, in exploratory work, we must use simpler techniques, such as (1) enzyme digestion (β -amylase), (2) crystallization methods, (3) adsorption on cellulose or other adsorbents, (4) determination of reducing power or alkali lability (aldehyde assay), (5) iodine absorption spectrum, (6) potentiometric iodine titration, (7) x-ray diffraction, and (8) viscosity and allied methods.

⁶ The crystallinity of retrograded starch is demonstrated by the fact that it gives a crystalline x-ray pattern of the B type. Under some conditions retrograded amylose is noticeably birefringent.

A. Hot Water Fractionation—By extracting swollen corn starch granules with hot water, Meyer found that 5 to 20% of the total starch could be leached out, and that on concentration the extract became insoluble (17). By chemical analysis of end-groups, the material was shown to be fairly pure amylose, *i.e.* starch with straight chains; the granule residues were shown to contain the branched molecules originally present in the whole starch.⁷

The mechanism involved in fractionation by hot water is the loosening of the granule structure and the extraction of molecules not too tightly bound or entangled in the network. The amylose molecules are able to diffuse slowly out of the swollen granules, while branching in the amylopectin molecules tends to cut down the rate of diffusion to practically zero. It is reasonable to suppose that the fractionation will not be a complete one, since some of the straight chain amylose molecules will be so hopelessly entangled that their rate of diffusion out of the structure will be very slow; on the other hand, many of the amylopectin molecules on the surface of the granule will be torn loose and will appear as an impurity in the amylose. For these reasons, it is advisable to purify the raw amylose by another fractionation technique.

B. Butanol Precipitation—A fundamentally new method of starch fractionation was discovered by Schoch during an investigation of the effect of alcohols on starch pastes (13). It was found that butanol and pentanol cause a selective precipitation of the amylose constituent of starch, leaving the amylopectin in solution (18). Although the raw amylose contains a considerable amount of coprecipitated amylopectin, by repeated crystallization the amylose becomes substantially free from branched chains. The non-precipitated fraction contains a small amount of amylose which gives this fraction a deep blue iodine color; however, on the removal of less than 10% of the material by adsorption on cotton (see below) the blue-staining characteristics are removed and pure amylopectin remains.

X-ray and optical analysis of the butanol precipitate has shown that the amylose chains form helical coils, presumably around the alcohol molecules, and the cylindrical molecules then pack closely with the formation of the characteristic rectangular platelets and hexagonal rosettes of the butanol precipitate. In amylopectin the frequent branching interrupts the regularity of the amylose chain, and the molecule is unable to participate in the helical cylindrical crystallization. The molecular weight of butanol-precipitated amylose is considerably higher than that of the corresponding amylose from hot water extraction (14); possibly this is because of the presence of an impurity of high molecular weight, but more likely because in the hot water extraction the amylose of high molecular weight is more slowly extracted because of its higher entanglement in the granule structure. Moreover, an impurity of very high molecular weight, *i.e.* amylo-

⁷ However, Meyer did not show that all the amylose had been extracted from the insoluble granule residues, and since higher yields of amylose have been obtained by other methods, it is

molecule per 8 glucose residues. At this point, the iodine activity rises more rapidly on the addition of iodine solution, however, not as fast as in potassium iodide solution alone. This rather abrupt change in the rate of increase of iodine activity at 18.7% absorption, followed by additional absorption, indicates beyond doubt that there are two distinct phases of the reaction: (1) Iodine is taken up isopotentially, combining with the amylose at a potential which is characteristic for the type of amylose present; and (2) increasing amounts of iodine are taken up at increasing iodine potentials. The first phase, during which the typical blue color is formed and gradually deepened as iodine is added, has many of the characteristics of a heterogeneous reaction; that is, the iodine activity is a constant independent of the amount of either amylose or the iodine addition product present. Following 18.7% absorption, the characteristics of the reaction indicate absorption at the surface of the molecules or the distribution of iodine between two immiscible solvents.

The potential at which iodine is taken up during the titration of an amylose or other starch fraction is a characteristic of the sample and is apparently a function of the chain length. Thus, corn amylose which has a molecular size of approximately 250 glucose residues takes up iodine at a somewhat higher potential than does potato amylose, the molecules of which are roughly twice as large. On the other hand, amylopectins, short straight chain amyloses of about 20 to 30 glucose residues, take up iodine at a much higher potential. In mixtures, the constituent which absorbs iodine at the lowest potential is titrated first, then the potential rises abruptly to the next lowest potential, and so on. This behavior is characteristic of heterogeneous systems.

Amylopectin fractions and glycogen do not take up iodine isopotentially, but at gradually increasing potentials somewhat as in the second phase of the amylose reaction. Moreover, the amount of iodine bound is much smaller than in amylose. Instead of a deep blue color, a reddish or brown color is developed, as is also the case with the amylopectins of low molecular weight. Raw starch or mixtures of amylose and amylopectin may be analyzed by potentiometric titration; the absorption of iodine by the amylose takes place as if no amylopectin were present, and the amount of amylose can be readily calculated on the basis of 18.7% iodine for pure amylose.

The behavior of whole starch during potentiometric titration indicates the presence of but two distinct fractions, amylose and amylopectin. All so called fractions which have properties intermediate between amylose and amylopectin are titrated as mixtures of amylose and amylopectin, and in most cases sub-fractionation has been successful. The potentiometric titration of starch with iodine then constitutes a powerful argument in favor of a two component theory for starch, as well as a useful tool for the analysis of whole starch and starch fractions.

C. Optical Properties of Amylose-Iodine Complexes—Extremely dilute amylose-iodine solutions exhibit very broad absorption bands with maximum absorption at about 600 $m\mu$, while with amylopectin or amylopectin solutions the absorption

maxima are shifted to shorter wave-lengths. The absorption in starch-iodine solutions is much more intense than that in solutions containing corresponding amounts of iodine in organic solvents.

Amylose-iodine solutions oriented during flow become quite dichroic, indicating an orientation of the iodine molecules (24). Since the velocity gradients in streaming fluids would scarcely orient iodine molecules, one must assume that the amylose molecules become oriented and in turn orient the iodine molecules. The dichroism indicates that the long axes of the iodine molecules are parallel to the direction of flow, while the highly asymmetric amylose molecules must also be oriented roughly parallel to the flow lines. These considerations, together with evidence from x-ray diffraction, indicate that in amylose-iodine solutions as well as amylose-iodine crystals the iodine molecules are tightly held in the center of a helical amylose coil. During flow, the helices orient themselves roughly parallel to the flow lines and thereby orient the iodine molecules.

On precipitation of starch-iodine, the helices pack together closely to give an organization which exhibits a V type x-ray pattern (11). The iodine molecules must be packed practically end to end if there is 1 iodine molecule for every 6 or 8 glucose residues. It is questionable whether a regular periodicity exists or whether the iodine molecules are packed more or less at random, but at any rate the molecules are held rigidly parallel to the helix axis. Possibly, the intense light absorption, characteristic of the amylose-iodine complex, is due to the combined effect of many collinear iodine molecules.

7. Optical Rotation of Starch. Most values for the specific rotation of starch range between $[\alpha]_D = +180^\circ$ and 220° ; in fact, there are so many different values in the literature that one is tempted to believe that each worker has his own method for determining the specific rotation of starch fractions. On the other hand, most values reported for hydrolytic dextrans of high molecular weight which are readily soluble lie between $[\alpha]_D = +190^\circ$ and 200° . The difficulty in measuring the rotation of undegraded starch arises from the opacity of the solutions used. For this reason it is the custom of many workers to measure the rotation of starch in alkaline solutions which are relatively clear. By dissolving amylose in alkali, neutralizing, and immediately taking the rotation, Meyer obtained a value of $[\alpha]_D = 220^\circ \pm 5^\circ$ (1). This value is significantly higher than the average of values reported by other workers for whole starch. The specific rotation of amylopectin may be taken as $[\alpha]_D = +200^\circ$; however, the high opalescence and opacity of aqueous solutions make this value somewhat uncertain. On the other hand, freshly prepared solutions of butanol-precipitated amylose give clear limpid solutions whose rotations can be measured almost as accurately as those of ordinary crystalline compounds. Using clear supernatants from freshly prepared amylose solutions, one obtains values of $[\alpha]_D = +200^\circ$ with an uncertainty of about 2° . The butanol-precipitated amylose contains a large amount of water and butanol of hydration; so it is impossible to make up the solutions accurately by weight, and there are usually some particles of amylose which are undissolved even after boiling several minutes. For these

reasons, it is necessary to determine the concentration of the solution used for the measurement of specific rotation by evaporating a small amount to dryness.

Since it is relatively simple to determine accurately the specific rotation of amyloextrins, as these compounds give water-clear solutions, it is possible to calculate a value for the specific rotation of an amylose of infinite chain length and hence that of an amylose of any chain length by means of the so called Freudenberg equation (25)

$$[M]_n = [M]_2 + (n - 2)[M]_{\infty/\infty}$$

where n is the number of glucose residues in the amylose chain, $[M]_n$ and $[M]_2$ are the molecular rotations of the n -membered amylose and maltose respectively, and $[M]_{\infty/\infty}$ is the molecular rotation per glucose residue of an infinitely long amylose chain. For example, an amyloextrin containing 22 glucose residues has a specific rotation of $[\alpha]_D = +193^\circ$, while maltose hydrate has a rotation of $[\alpha]_D = +131^\circ$. The corresponding molecular rotations are then $[M]_{22} = 691,300$ and $[M]_2 = 47,200$, and the molecular rotation per glucose residue is $[M]_{\infty/\infty} = 32,200$. This corresponds to a specific rotation for the infinitely long amylose of 199° , which is in excellent agreement with the above value obtained from butanol-precipitated amylose.

As the value of the specific rotation of amylopectin is also very close to $[\alpha]_D = 200^\circ$, small amounts of amylopectin will not influence the value obtained for the specific rotation of amylose. Since amylopectin has an irregular structure and is rather difficult to work with in the polarimeter, comparatively little is known about the amylopectin dextrins. For the present, one may assume that amylopectin dextrins have roughly the same specific rotation as the corresponding amylose dextrins.

8. Other Aspects of the Colloidal Behavior of Starch. The sizes and shapes of homogeneous colloidal molecules may be determined from the results of ultracentrifugal measurements together with data on the rate of diffusion, partial specific volume, and viscosity of both solution and solvent. As yet comparatively little has been published concerning these relatively important phases of the starch problem. A complete investigation of the sedimentation and diffusion of glycogen by Bridgman (26) indicates what may also be expected of homogeneous starch fractions. He found that five samples of glycogen were quite highly polydisperse, with average molecular weights ranging from 4 to 14 million (corresponding to molecules containing 25 to 90 thousand glucose residues). The experiments indicate an unexpectedly high asymmetry for glycogen, which is usually considered to be roughly spherical; indeed an axial ratio of 18:1, or higher, was found to exist.

An ultracentrifugal investigation of whole ground corn starch by Beckmann and Landis (27) indicates that the starch is decidedly heterogeneous and consists of two main fractions. The heavy component sediments very rapidly, having a sedimentation constant of approximately 6000 S , while the lighter component has an average sedimentation constant of 4 S with considerable spread of S

values. Rough diffusion constants were also determined from the ultracentrifuge diagrams and were used to calculate molecular weights and asymmetry ratios. These authors estimate the average molecular size of the amylose fraction to be approximately 300 to 400 glucose units, while the amylopectin fraction is thought to be about 1000 times larger. The axial ratio of amylose, estimated from the f_0/f_∞ values given, ranges from 2.5 (almost spherical) to about 45.

A similar investigation of potato starch by Coles (28) showed that potato amylose consists of molecules of higher average molecular weight and of considerably higher asymmetry than corn amylose. The average molecular weights given by Coles for potato amylose and amylopectin are 185,000 and 1,000,000 respectively. After degradation by grinding or enzyme action the molecules become much smaller and less asymmetric.

It must be pointed out that while it is possible to determine diffusion constants of homogeneous compounds of high molecular weight in the ultracentrifuge, the values obtained in this way from heterogeneous preparations and compounds with long molecular chains in particular are not generally acceptable. The difficulties of maintaining starch preparations, especially amylose, in aqueous solution without aggregation or degradation also complicate the problem.

Earlier sedimentation experiments on starch are now generally considered unreliable, inasmuch as the samples were mixtures of unknown proportions and moreover were often highly degraded. The difficulty of maintaining types of starch in a condition of complete dispersion without degradation has been solved by the use of such solvents as chloral, hydrazine, ethylenediamine, and others; we may presently expect more reliable reports on the sedimentation and diffusion of starch components.

The high viscosity of completely dispersed starch solutions must be interpreted as a strong indication that the molecules are, on the average, highly asymmetric. On the basis of studies by Staudinger, average starch molecules are appreciably less asymmetric than cellulose molecules of the same molecular size (29). Although Staudinger did not have access to the newer starch fractions, it is also true that even the best amylose preparations do not cause as much increase in viscosity as corresponding cellulose solutions (30). This is due, in part, to the difference in the configuration of the glucosidic linkage and to the tendency of the starch chains to form helical coils, both of which cause a considerable shortening of the molecule.⁸

On treatment with small amounts of sodium chloride, the viscosity of amylose solutions is reduced to 10% or less of its original value (1). Since sodium chloride cannot be considered a degrading agent, it must be due to a change in the configuration of the molecule. One may readily calculate that a change from the extended configuration to the helical would cause this much change and more, but as yet there is no direct evidence that such a change takes place.⁹ It may

⁸ See the x-ray studies above.

⁹ For example, consider a rigid amylose molecule containing 200 glucose residues. When fully extended, the molecule would be roughly 1000 Å. long and 6 Å. in diameter with an axial

be said, however, that the high intrinsic viscosity of amylose solutions in pure water is not indicative of a compact helical configuration.

One must not confuse the high viscosity of starch pastes with that of the corresponding well dispersed constituents. Microscopic examination shows that in freshly prepared pastes there are multitudes of swollen granule residues which tend to adhere to each other and which thereby increase the apparent viscosity of the solutions. In order to obtain estimates of the asymmetry of molecules from viscosity or diffusion methods, it is basic that the substance must be in a condition of complete dispersion.

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ratio of 167 : 1; in the helical configuration the molecule would be 250 Å. long and 13 Å. in diameter, with an axial ratio of about 19 : 1. The corresponding viscosity increments are (31): extended configuration, *ca.* 1500, and helical configuration, *ca.* 35. A 10-fold decrease in viscosity would result if 78% of the amylose was converted from the extended to the helical configuration. Flexible molecules would be less highly asymmetric but the change after coiling would be similar.

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CHAPTER VIII

CHEMICAL PROPERTIES OF STARCH COMPOSITION AND STRUCTURE

1. Introduction. The starches are hydrolyzed by dilute acid almost completely to glucose. They are split by diastases to the disaccharide maltose in yields approximating 80%; the 20% balance, consisting of material of higher molecular weight, can be hydrolyzed with dilute acid or with α -glucosidases to glucose. Minor amounts of non-carbohydrate residues are always obtained following hydrolysis, and are composed of fatty acids, other lipids, substances of protein origin, and inorganic salts, in percentages which vary with the origin of the starch. The empirical formula for starch is, therefore, represented as essentially an anhydride of glucose. Most of these glucose units might be presumed to be joined as in maltose. The main problems relating to starch composition and structure are (a) to establish by what type of linkages the glucose units are united to form the component molecules, (b) to determine whether one or more molecular species occur in any one starch, (c) to learn whether the same structure or structures occur in all starches, and, if the starches are found to be chemically heterogeneous, to determine the relative proportions of the various structures present, and (d) to evaluate the small quantities of non-carbohydrate constituents present in each starch.

Nearly 130 yrs. have elapsed since de Saussure (1) correctly interpreted the result that glucose is produced from starch by a hydrolytic reaction. It is well over a century since this same investigator isolated maltose as an intermediate in the hydrolysis of starch (2). Nevertheless, the development of our knowledge of starch structure has been surprisingly backward, when comparison is made with the development of the organic chemistry of some of the other substances of biological origin. Some phases of the problem still require further study. An analysis of the early literature is instructive, in that it brings to light several reasons which explain the retarded development of this branch of carbohydrate chemistry. Some of these involve errors, both of omission and commission. Inasmuch as several of these errors have been repeatedly made in different periods of the development of the subject, indeed some still persist in what might be classed as current literature, it would seem desirable, in this effort to lay the foundation for a completion of the problem, to review and point out the significance of the more important reasons found. The following are given, although not necessarily in the order of their importance: (a) For more than

50 yrs., the full significance of de Saussure's important observations, mentioned above, were not appreciated. Indeed, as late as 1872 it was generally believed that the diastases hydrolyzed starch to glucose, rather than maltose. (b) The delay in deducing the correct structures for both glucose and maltose by the sugar chemist has naturally retarded the starch chemist in his determination of how these units are joined together in starch, for as late as the nineteen twenties it was generally believed that the principal glucosidic linkage in starch was from aldehydic carbon atom 1 of one glucose unit to carbon atom 5 of the next. This view would seem less likely today, since carbon atom 5 of glucose is now believed to be a part of a pyranose ring and hence the possibility of a glucosidic union through this carbon would be less. (c) Many studies on the constitution of the starch molecule have proceeded without due regard of the possibility that the starches may be chemically heterogeneous. (d) There has existed, too frequently, a failure to appreciate that starch or certain starch components are very labile, not only in the chemical sense, but what is apparently of equal importance, in respect to physical properties. Not only is this class of components metastable themselves, but they appear to be able to induce this characteristic in mixtures of which they may constitute but a small fraction of the total. (e) This physical instability and the mutual effect of one component on the physical behavior of another have made a complete separation of the individual starch components (or even a division into molecular species) very difficult. Indeed, it would seem that only within the last year or two the major starch components had been isolated in a degree of purity consistent with good practice in organic chemistry. (f) It has been assumed, in some cases, that all starches are very nearly the same, or stated differently, that the experimental data obtained from the study of one starch, *e.g.* potato starch, could be used to formulate a theory to explain the behavior of another, *e.g.* wheat starch. Additional reasons will be given in the following review of some of the early work in starch chemistry to illustrate the above discussion.

In their early reports, Maquenne and coworkers have discussed many of the problems involved in fundamental starch chemistry. For example, Maquenne has given an unusually clear account of the physical lability of one component of the starches, subsequently called amylose. This phenomenon he termed retrogradation (3-5) and proposed that it was possibly of the nature of a crystallization involving an association of the amylose molecules. Because of this behavior, amylose (or amylocellulose, as it was once called) is capable of existing in a variety of physical forms, showing all gradations between a highly water-soluble state and one completely insoluble. It is to be noted that Maquenne did not differentiate chemically between those types of amylose which are originally insoluble in hot water and those that are originally soluble but which may become permanently insoluble under certain conditions. On the basis of the observations mentioned, Maquenne rejected the prevailing concept of that period, that the components of starch could be differentiated solely on the basis of solubility or insolubility in water.

He did, however, subscribe to the theory of starch heterogeneity, which had appeared in many forms since Leuwenhoeck, in 1716, observed that starch granules appeared to consist of two components, an inner, digestible fraction, soluble in hot water, and an outer, less digestible, hull-like material, insoluble in water. Maquenne proposed that a chemical difference existed between the components, which difference explained the results of diastatic hydrolysis.

Brown and his coworkers (6, 7) had proposed that maltose originated from one part of starch and that the other main product of diastatic hydrolysis, the resistant limit dextrins, were formed from another. However, these workers had assumed that starch was composed of only one type of molecule. This was thought to consist of a union of two different radicals, maltan and dextran, in the ratio of 80 : 40. Maquenne's principal contribution was the separation, essentially by physical means, of a component which he claimed was completely converted to maltose by diastase. This important finding, that a difference in chemical structure exists and that one component is formed exclusively of maltose residues, was soon forgotten or ignored. An unjustified inference from this experiment, that all the maltose produced in diastatic hydrolysis originates from this starch component alone, persisted for many years.

Maquenne obtained crude solutions of amylose without what is termed paste formation of the starch. Furthermore, purification of the product by allowing it to retrograde, or spontaneously precipitate, redissolving in water at high temperatures, and repeating these processes several times gave, finally, a substance which did not gel but rather precipitated in a powdery or granular state. It might be reasoned that, if starch is heterogeneous, the number of components must be limited to two; then the conclusion that the property of paste formation is a characteristic of the component which is converted into dextrins by diastase follows. To this hypothetical component Maquenne applied the name amylopectin (8).

The terminology suggested soon found wide acceptance and is in use today. Very little was done, however, to expand his findings. Instead, and possibly because Maquenne subsequently wavered in his own conclusions (9), various interpretations of his theory have been given and various definitions of amylose and amylopectin have been substituted for the original ones. The result has been that as fractions of starch have been separated from time to time, by a variety of methods and in undetermined states of purity, they have been arbitrarily called either amylose or amylopectin, on the supposition, no doubt, that, if they were not one, they must be the other.

Added to this confusion has been the persistence of some to employ an older terminology, outmoded by the work of Maquenne; *i.e.*, α -amylose to denote an insoluble fraction of starch and β -amylose to indicate a soluble fraction. Some workers (10) evidently saw a relationship between the two concepts and used the term α -amylose synonymously with amylopectin. Others (11) have used the term β -amylose for fractions that were predominantly of an amylopectin character.

Of the methods proposed to separate amylose from amylopectin the following are more frequently encountered in the literature.

Amylose has been prepared by freezing starch pastes for several hours, thawing, and extracting the amylose with hot water (12). Obviously very little fractionation should result with starches which contain an easily retrograded amylose. Indeed, with starches such as corn, it is certain that the final water extract should be richer in amylopectin than in amylose.

Some workers (13) have attempted to purify the amylopectin in the frozen waddy-like mass by treating the latter with a diastase and washing. This treatment should insure that the product is relatively free from amylopectin. The insoluble residue would certainly not be expected to be free from retrograded amylose.

Salts of the alkaline earths have been used to precipitate amylopectin from starch pastes (14). Actually, calcium and barium facilitate the precipitation of amylose from whole corn starch pastes.

Electrophoresis or electrodecantation (15) has been used to separate potato amylopectin. This was claimed to be possible by virtue of its esterified phosphate radical. The method applied to native corn starch, after gelatinization, yields, however, small amounts of retrograded amylose types in combination with, or adsorbed to, fatty material. Certainly the material remaining in solution is no more pure in respect to amylose than is the original whole starch. The insoluble product from the fractionation has been called α -amylose in the many researches on corn starch by T. C. Taylor and as so used is not, therefore, synonymous with amylopectin.

One of the earliest methods given for preparing amylopectin is to dissolve starch in sodium hydroxide, neutralize, dilute to a 0.5% paste, and allow to stand. The precipitate which forms, instead of being amylopectin, as claimed (16) is a mixture of retrograded amylose and small amounts of amylopectin.

Hot water extraction of starch and even the purification of the extracted amylose by retrogradation have not been completely overlooked, as one might infer from recent dissertations on the subject (17). Both Tanret (18) and Baldwin (19), among others, made a serious effort to use and improve on the method. Several difficulties inherent in the method will be discussed in greater detail in the sections that follow. First, it has been repeatedly noted that as the intensity of the hot water extraction is increased, in order to obtain appreciable yields of amylose, the purity of the extract, in respect to amylose, becomes less and less. When considerable quantities of amylopectin also dissolve, Tanret recommends further purification by adsorption of the amylose on cellulose fibers. Retrogradation of the amylose from solution, to purify it, was suggested by Maquenne. However, unless the spontaneous precipitation is repeated many times, it is doubtful whether a material purification is effected by such a procedure, because the retrograding types of components can either induce a similar effect in the other types or else they can carry down with them substantial quantities of the non-retrograding components. This fact is obvious by so simple an

experiment as allowing a 5 to 10% paste of corn starch to age. After it has stood, it would appear that practically all of the starch had retrograded to an insoluble or non-reversible gel. Furthermore, the method of hot water extraction fails to take into account that constituents other than amylopectins may be originally water-insoluble. Yet it is certain from the early works of Gruzewska, Schryver, Ling and Nanji, and others that a component exists in some starches which is originally quite insoluble in water, which is not an amylopectin in character, but which is related to the amylose type of components. After treatments which tend to solubilize it, it is stained an intense blue by iodine and is converted to maltose by diastase.

Kerr and coworkers (20, 21) have discussed the efficiency, or rather lack of efficiency, in separating starch components by the general methods mentioned above. It is little wonder that the amylose (or amylopectin) content for any one starch will be found reported in the literature to be within the entire range from practically zero to 100%, depending on the method of estimation used (22). It is therefore understandable, perhaps, that modern chemists, confronted with such conflicting data and divergent theories of starch composition, should have either ignored the heterogeneity of the starches or else should have sought to show that amylose and amylopectin were chemically identical (23).

Once again, however, the heterogeneity of the starches seems to have been reestablished, mainly through the efforts of Hess, Samec, K. Meyer (17), Pacsu (11), Schoch (22), Kerr (24, 20, 21), and their coworkers. It should be borne in mind, none the less, in tracing the development of the modern concept of starch that for the greater portion of the last decade this theory was generally denied and starches were treated as though they contained but one single type of molecule.

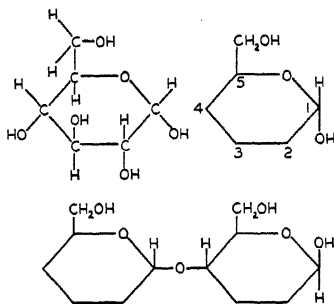
2. Development of Modern Concepts of Starch Structure. The development of the modern concept of starch structure probably starts with the work of Haworth during the early thirties. It would seem advisable, in order to obtain a proper perspective for present theories and research, to review more critically the work that has been reported during the last 10 yrs.

The principal analytical tool used by Haworth, Hirst, and others of this period has been termed exhaustive methylation. This procedure involves methylating the starch with methyl sulfate, methyl iodide, or a similar reagent, usually repeatedly, so that finally each free hydroxyl group present in the original substance is derivatized into a methyl ether group. Inasmuch as ethers are fairly resistant to hydrolysis by acids, the methylated carbohydrate may then be degraded to methyl sugars, with all the methoxyl groups intact. Finally, the resulting methylglucose or methylglucoses are examined and any of the 6 carbon atoms in glucose found to be without a methoxyl group is assumed to be either a point linking the various glucose units together to form the original carbohydrate or else the carbon which takes part in ring formation.

Irvine had applied this method, a decade earlier, to elucidating the structure of simpler carbohydrates and also of starch. From the latter he obtained as

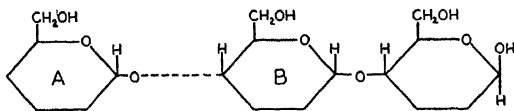
the final product 2,3,6-trimethylglucose. But, in accordance with the prevailing concepts of that era, he deduced that the glucose units in starch are joined one to the next by a 1-5 glucosidic linkage and that each glucose contained a furanose ring, that is a hemiacetal bridge from carbon 1 to carbon 4. 6 glucose units so linked were then thought to form a closed ring or hexagon (25).

Haworth's work would indicate that glucose exists predominantly in the pyranose form with an oxygen linkage between carbons 1 and 5. In applying the methylation procedures to maltose, he obtained both 2,3,6-trimethylglucose and 2,3,4,6-tetramethylglucose and assigned to this sugar the now accepted structure (I) (26). Extending his technique to starch, he obtained, in addition



I. Upper row, structure of glucose, simplified form of notation given at the right; lower row, structure of maltose.

to 2,3,6-trimethylglucose, 4.5% of 2,3,4,6-tetramethylglucose. From this result, Haworth proposed that the glucose units in starch were joined as in maltose (II).



II. The chain structure proposed for starch. The "A" unit yields 2,3,4,6-tetramethylglucose; the "B" units yield 2,3,6-trimethylglucose.

For every 20 to 25 glucopyranose units present in the chain (as that glucose unit designated B) which would yield 2,3,6-trimethylglucose, there is present 1 glucopyranose unit (designated A) which would yield 2,3,4,6-trimethylglucose on methylation and hydrolysis. Haworth deduced from these results that the minimal length of the chain is approximately 24 to 30 glucose units and the chain has a molecular weight of about 5000 (27, 28, 23).

The experiments of Haworth were extended and the exhaustive methylation technique was applied to the study of various starches and starch fractions by many workers. Preparations purported to be amylose and amylopectin were methylated and found to give identical results, from which it was concluded

that the essential difference between them lay in their state of aggregation into supermolecular units (23, 29). The chain length of the molecules in each case was estimated at 24 to 30 glucose units. In addition to potato starch, maize and waxy maize were found to have molecules averaging 24 to 30 glucose units in length also (30, 31). Hassid and Dore (32) reported that the Haworth configuration could be applied to *Canna* starch. Only glycogen ("liver starch") was found to give substantially different results, from which the minimal length was estimated to be between 12 and 14 glucopyranose units (33, 34). The configuration, however, was reported to be the same as for the vegetable starches. It appeared, in 1935, that the main features of the problem concerning the structure of starch had been solved (29, 35).

So simple a concept for the composition and structure of the starches fails to explain adequately many observations in respect to their behavior. If the possible heterogeneity of the starches is disregarded, a structure consisting of a straight chain of 24 to 30 glucopyranose units is not in harmony with (a) the very feeble reducing power of starch, (b) the apparent enormous size of starch molecules in solution, as evidenced by viscosity, osmotic pressure, and sedimentation experiments, and (c) the results of the action of the diastases on the starches. Haworth has pointed out these incongruities and assumed at first that the unit chains were aggregated into a unit of larger magnitude in a manner which would mask the reducing power of the terminal aldose groups. As a basis for explaining the diastatic degradation of starch, Haworth (36) showed, using the methylation technique, that the dextrins remaining after the action of barley diastase on starch have a chain length of 12 glucose units. This is approximately 40% of the 24 to 30 glucose units estimated as the basic chain length of the parent starch molecule. The result agrees with the observation that barley diastase converts about 60% of starch to maltose.

Hanes (37) has prepared an extensive review of the biochemistry of starch, which in general supports the views of Haworth. To explain the cessation of activity of β -amylase, when only 60% of a supposedly uniform chain has been hydrolyzed, Hanes proposed that a terminal section of each chain, some 40% of the molecule, is involved in a supermolecular aggregation. This makes impossible the proper approach of the enzyme. The balance of the molecule does not aggregate and is therefore vulnerable to attack by the enzyme.

Myrbäck (38), however, could not support the view that the limit dextrins that remain when β -amylase acts on starch are of low molecular magnitude. In contrast to the bodies of low molecular weight which result when α -amylase hydrolyzes starch, some of those resulting after β -amylase activity are of almost the same order of magnitude as starch itself. The results are calculated from osmotic pressures of the dextrins and starch. Myrbäck concludes that enzymic activity is retarded because certain anomalies exist in the configuration; that is, starch is not composed exclusively of maltose residues. Hence, since only a maltose configuration fits the structure of the β -amylase, when a configuration is encountered in the degradation of the starch molecule which is not maltose,

activity ceases or is greatly retarded. The conclusion is substantiated by further degradation of these dextrans by taka-diasase to sugars of low molecular weight with a chain length as short as 3 glucose units and by showing that these final products are not fermentable.

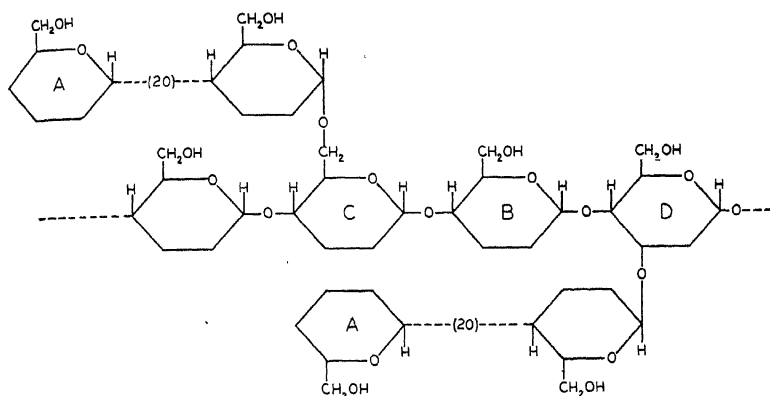
Richardson and coworkers (39) developed a technique for measuring the reducing power of starch by using copper in a slightly alkaline solution. The results obtained led Richardson to conclude that the chain length of starch averaged about 1000 glucopyranose units. He also pointed out that if acid acts at random on starch it requires the cleavage of only a very small percentage of the glucosidic bonds in the chain to reduce the average length to that of the unit chain found by Haworth. Inasmuch as Haworth's methylation procedures involved acetylation of the starch as a preliminary step, with sulfur dioxide and chlorine as the catalyst, Richardson finds that Haworth's estimate of chain length is open to criticism.

Richardson's criticism of Haworth's work led to several very interesting consequences. First of all, Haworth repeated his procedures, but acetylated the starch in pyridine with acetic anhydride, and claimed that practically no degradation occurred in the process. He showed, moreover, that the amount of tetramethylglucose formed after methylation and hydrolysis was approximately the same as that he had obtained in his earlier work, by acetylation in the presence of SO_2Cl_2 , a rather surprising result. Richardson and his coworkers (40) repeated the experiment and found that their copper reduction values did not increase after the starch was acetylated in pyridine and the acetate saponified with dilute sodium hydroxide, thus confirming the result of Haworth's experiment. However, the results of Richardson and coworkers may be taken to support the view that starch is composed of macromolecules (rather than aggregates of small molecules) whose molecular weight is many times larger than that of the unit chain postulated by Haworth.

Haworth, Hirst, and Isherwood (41) exhaustively methylated rabbit liver glycogen and obtained an amount of dimethylglucose which was about equivalent to that of the tetramethylglucose isolated as the end-group. They significantly drew attention to the fact that dimethylglucose may be found in the hydrolysate of methylated starch and proposed that the unit chains of both glycogen and starch are linked either by a covalency bond produced by dehydration or by a coordinated or hydroxyl bond which links the reducing end of one chain with another glucose unit of an adjacent chain.

From essentially physical measurements, Staudinger (42) came to the conclusion that Richardson's estimate of molecular magnitude is substantially correct. From measurements of the viscosity and osmotic pressure of starch solutions, Staudinger finds about 1500 glucose units to the starch molecule. Furthermore, the fact that the same values are obtained for specific rotation as well as viscosity and osmotic pressure, respectively, before acetylation and after saponification of the acetate, is given as conclusive evidence that macromolecules and not aggregates are involved. Staudinger then concludes that these macro-

5 molecules appear to be highly convoluted and proposed a symmetrically branched structure for the configuration of the starch molecule as shown here (III).



III. Starch structure according to Staudinger (1937). The "C" units yield 2,3-dimethylglucose; the "D" units yield 2,6-dimethylglucose.

By reference to this formula it will be seen that the results of both Haworth and Richardson are harmonized. If branching through primary valence linkages does exist, then it is possible for the structure to contain 1000 or more glucopyranose units, and yet the ratio of tetramethyl- to trimethylglucose formed may be the value found by Haworth; that is, the ratio (given as 1 : 23) of terminal A units to intermediate B units will be maintained.

The branching denoted is through glucosidic linkages, and, if this branching is symmetrical as proposed by Staudinger, first through a carbon-6 on one side of the chain (as in unit C) and then through a carbon-3 on the other side of the chain (as in unit D) and if such a structure is methylated and hydrolyzed, then in addition to tetra- and trimethylglucose one should obtain certain amounts of 2,3-dimethyl- and 2,6-dimethylglucose. Staudinger pointed out that quantitative data needed to confirm the configuration predicted were yet to be supplied.

Freudenberg and Boppel (43) methylated starch directly in liquid ammonia with sodium and methyl iodide. After several successive treatments a product was obtained which showed 45.5% methoxyl groups on analysis. Although substantially the theoretical value was obtained for a fully methylated starch, assuming a chain of indefinite length, dimethylglucose was obtained after hydrolysis. Further work disclosed that both 2,3-dimethyl- and 2,6-dimethylglucose were present.

Confirmatory evidence for a branched structure was furnished by Myrbäck (44) who extended his studies on the end-products of the hydrolysis of starch and isolated a trisaccharide in which one of the carbon-6 hydroxyls was not free. From this he concluded that the trisaccharide isolated contained a 1-6 glucosidic

linkage. The inference is that this product represents the residue of a branched portion of one of the original starch molecules.

Other workers, in the meantime, *e.g.* Hess and coworkers and Hirst and Young (45), had taken up the question of a branched chain structure for starch. Haworth (46) concluded that the polymeric link which joins the unit chains together in starch is a glucosidic, primary valence. In a recent communication, Haworth (47) emphasizes that neither he nor his coworkers had proposed that the unit chain length is identical with the molecular size of starch, but rather that it represents repeating units composing a much larger structure. The report of Bawn, Hirst, and Young (48) is referred to as evidence that the repeating units are joined through primary valences, and the work of Barker, Hirst, and Young (49) is given to support the view that these linkages are glucosidic unions through the primary alcohol groups on carbon-6.

Certain limitations have developed, however, in the use of methylation data for deducing the configuration of starch. Further work by Freudenberg and coworkers (50, 51) requires that views be modified as to the extent and type of branching in starch. Although repetition of the experiments again disclosed the presence of both 2,6- and 2,3-dimethylglucose derivatives in a mixture of products from methylated starch, it was found that the former can and does quite likely result from the extended hydrolysis of the primary product, 2,3,6-trimethylglucose. Hence it is thought that branching is limited only to side chains connected through 1-6 glucosidic linkages, rather than the symmetrically branched configuration proposed by Staudinger, with a 1-6-linked branch on one side of the main chain, followed by a 1-3-linked branch on the other.

Subsequently, Freudenberg's methylation technique, among others, has been criticized by Meyer (52), in that methylated starch of relatively low viscosity is produced. To Meyer, low viscosity is evidence of a degradation in starch structure during methylation. Obviously, if such degradation is the result of hydrolytic cleavage, as Meyer contends, new terminal glucose units which are not present in the original starch are thereby produced. But it should be pointed out that even after an exhaustive treatment Freudenberg finds only 3 to 4% of tetramethylglucose to result from whole starch, which is substantially the same value obtained by Meyer at a definitely lower degree of methylation.

On the other hand, as intimated by Haworth (47), a criticism of Meyer's work might be made on the ground that he had attempted rough calculations of end-groups from incompletely methylated products. Also one may use Freudenberg's data to show that this might not be a safe procedure, for whereas in earlier work Freudenberg (53) reported finding only 1% of tetramethylglucose when starch was short of being completely methylated by about 1% methoxyl content, or 44.5% methoxyl, the later paper of Freudenberg cited reports 3 to 4% of tetramethylglucose to result when the methylation is carried approximately to completion. Hence, methylation and analytical techniques which result in the finding of 4% of tetramethylglucose when methylation is 2% short of completion are, rather, procedures that should be viewed with suspicion.

An equally serious matter, moreover, is the disturbing report of Caldwell and Hixon (54) that, in applying the methylation technique of Freudenberg and Boppel, they were only able to find about 1% of dimethylglucose in the hydrolysis products of fully methylated starch and less than this amount in the hydrolysis products of the methylated limit dextrins obtained from a soy bean, β -amylase conversion of starch. An analysis of methylated glucoses after a fractionation based on differential solubilities of the products was made by the method of Bell (55).

Hess and Lung (56, 57) believe that much of the methylation data reported are unreliable, in that starch suffers an alkaline degradation during methylation in sodium hydroxide solution possibly of the type reported by Taylor and more critically studied by Evans and by Schoch (58, 59). Hess and Lung methylated starch in an atmosphere of hydrogen and also, after partial methylation with dimethyl sulfate, they completed the methylation by their anisole method. They obtained a yield of only 1.9% of tetramethylglucose. Moreover, these workers produced methylated starches of various and widely different viscosities, all of which gave the same percentage of tetramethylglucose, about 1.9%. They are led to believe that a different degree of aggregation of the starch molecules was present in the various samples. Finally, the actual worth of all methylation experiments and data to that date is made questionable, in that their results do not permit them to exclude the possibility that the tetramethylglucose results more from one component of starch than from others.

In a subsequent publication, Hess and Krajnc (60) expanded on the last possibility and succeeded in demonstrating by methylation that there is a very decided difference in the end-group analyses of different starch fractions. This result is contrary to the views that had prevailed during the years just preceding. Comparing their starch fractionation technique with that of Samec and Haerdtl (15), they concluded that there are 10 times more end-groups in fractions predominantly amylopectin, than in fractions predominantly amylose. Electro-sedimentation of dispersed potato starch was the method used to separate the amylopectin fraction in the potato. Hess and Krajnc reported that 4.9 to 5.0% of tetramethylglucose results from the methylation and hydrolysis of their amylopectin fraction, whereas only 0.47 to 0.50% results from their amylose preparations. Hence, there is only one terminal group per chain of about 238 glucopyranose units in the amylose, whereas there is one terminal group per 23 glucopyranose units in the amylopectin.

Moreover, there is no relation between the viscosity of the methylated fractions and the chain length by end-group analysis or by observations on osmotic pressure. From the osmotic pressure determinations it is evident (61) that the molecular weight of the amylopectin preparation is very many times larger than the unit of the chain, and Hess concludes that this difference can be explained by assuming a branched chain structure. Some branching may exist in the amylose fraction.

Almost simultaneously, Meyer, Wertheim, and Bernfeld (62) reported a difference in the per cent of tetramethylglucose obtained by applying a methylation technique to hot water-soluble and insoluble fractions of corn starch. From the former Meyer and coworkers obtained 0.32% of tetramethylglucose, and from the latter 3.7%. These values are in fair agreement with those of Hess on potato starch fractions, but Meyer finds only one end-group per molecule for the amylose fraction, and in a note addressed to Hess, Krajnc, and Steuer, Meyer (63) insists that amylose is unbranched.

3. Starch Composition. Several laboratories have devoted serious thought during the period of studies on molecular structure to the separation of fractions of starch in attempts to isolate the components in states of purity consistent with good practice in organic chemistry. Mention has already been made of the work of Samec and of Taylor. From their many contacts with problems of starch chemistry, Kerr and coworkers were unable to accept the theory of chemical homogeneity, because it does not harmonize with the observed behavior of the starches, nor does it provide a basis for explaining the obvious differences between certain starches of different origin. Finally, Kerr and Trubell reported their work (24) in which the amylohemiacellulose of Ling (64) was further purified and studied. The isolated amylose was found to be substantially free from silica, but otherwise its properties were unchanged. It could not be therefore simply a silicic acid ester of amylose, as concluded by Ling. Samec had previously concluded that the presence of silica was not significant. An identical product apparently does not exist in starches from potato and tapioca.¹ The properties of this corn starch component, particularly its pronounced tendency to precipitate completely from dilute solution, and in more concentrated solutions to set to opaque and irreversible gels, parallel the most obvious and characteristic colloidal properties of corn starch and are in contrast to those of potato and tapioca starches. Although at first it was thought to be otherwise, it was subsequently found to be one of the more linear fractions of corn starch.

¹ Hudson *et al.* (65) recently have taken exception to this conclusion and to the implications which follow, since by the addition of 1% of oleic acid to potato starch they obtain 18% of insoluble material after a *Bacillus macerans* enzyme conversion of the mixture, and by extensively defatting corn starch before the enzyme conversion they reduce the insoluble fraction which forms from 10% to a range of 0.1 to 4.8%. However, the author is aware that the higher fatty acids are general precipitants for the linear polymers of starch, and Hudson does not compare the properties of the insoluble material obtained from the two starches to support his exception nor to support the implication that starches are mixtures comprised of only 2 component molecules. There is no longer any serious doubt that the "linear" fraction of potato starch differs from the "linear" fraction of corn starch. This conclusion was indicated by Kerr and Severson (66) and has been confirmed by Foster and Hixon (67) and others. In view of the experiments reviewed in the later part of this section, neither should doubt exist that the "linear" fraction of corn starch originally insoluble in water at 90° C. (and referred to previously as γ -amylose, after a purification involving degradation of the solubilized starch components by β -amylase) differs from the fraction extracted from starch by hot water (termed amylose by Maquenne and many subsequent workers). It is possible, however, that these differences are in respect to chain length only. This possibility is considered in the discussion which follows.

Later, Kerr and coworkers (20) pointed out possible errors in previously given concepts of starch composition based on products obtained by faulty methods of fractionation. These methods included hot water extraction, freezing and thawing of starch pastes, electrosedimentation, and the precipitation of amylopectins by the addition of alkaline earths. In no case is a separation of amylose from products of the amylopectin type obtained with the starch used. Electrosedimentation was applied to corn starch to precipitate the amylopectin-like material, a method which has apparently been used with some success with potato starch. From the data given, it would appear that a measurable fractionation of amylose is obtainable by an extraction of corn starch with water at 70° C., although yields are low and purities are of the order of 70 to 80%.

Meyer (68) had also sought to revive interest in the amylose-amylopectin theory of Maquenne. By a hot water extraction of starch and from essentially physical and biochemical studies on the extract and residue, Meyer had proposed that amylopectin differs from amylose in that its molecular weight is much larger and that its structure is branched.² In a series of subsequent publications, Meyer and coworkers claim to confirm the critical experiments of Maquenne by showing that a hot water treatment of corn starch will yield a solution from which the extracted amylose will spontaneously precipitate (17), and that, as so purified, the product after resolubilizing can be converted rather completely to maltose by a diastase, β -amylase. Further support to the conclusion that the fraction consists exclusively of maltose residues, *i.e.* that the structure is not branched, is afforded by the low yield of tetramethylglucose obtained after methylation and hydrolysis, a ratio of about 1 per molecule, as stated above (62). After a fractionation of this water-soluble portion, Meyer, Bernfeld, and Wolff (69) support the view advanced by Tanret (18, 70) that the amyloses are mixtures of a homólogos series.

The balance of the starch, Meyer finds as did Maquenne, is converted to a much lower yield of maltose, indicative of a difference in structure. It gives a much higher yield of tetramethylglucose per molecule. The branched structure postulated for starch by many workers, as already reported, is believed to pertain only to this fraction. From the work of Freudenberg and of Myrbäck, it is concluded that branching is through a 1-6 α -glucosidic linkage (71, 72). Sub-fractionation of the amylopectin fraction gives amylopectins of various molecular weights, but in all cases, according to Meyer, the branched pattern is the same; that is, there are no differences in the degree of branching. Contrary to the concept that the pattern of branching consists of a central chain with relatively side branches, Meyer proposes a highly ramified structure.

Haworth (47) has commented on Meyer's methylation data. In view of this criticism and in view of the work of Kerr and coworkers, which would indicate that the amylopectins are not so readily separated from amyloses by hot water

² Meyer reported that acetylamylose has a viscosity in chloroform comparable to acetylcellulose of the same molecular weight, but that amylopectin has only about one-fifth the viscosity of acetylcellulose of corresponding molecular weight. Later these statements were modified.

extraction of the latter, the task of isolating these amylopectins from the insoluble material being scarcely less difficult than from starch itself, the conclusion that two and only two structural patterns exist in starch (branched according to one definite pattern and unbranched) would seem to require further support.

Meyer (17) has presented two general schemes for obtaining subfractions of amylose. One is by increasing the temperature of starch extraction in steps, over extended periods of time, and results in a total of 31.2% of the starch being extracted. Quite obviously, of the four fractions described, at 70°, 70–80°, 80°, and at 80–90° C., the last two contain substantial quantities of amylopectin, as evidenced by a marked drop in the extent of conversion to maltose by β -amylase. In the second method extraction at 70° C. is employed and only about 6% of the starch is extracted. This material is subdivided into four fractions according to their insolubility in water on standing. The weights of the fractions obtained from 750 g. of corn starch are shown in Table VIII.

TABLE VIII

Yields and Molecular Weights of Fractions of Corn Amylose, According to K. Meyer

Fraction No.	Weight of fraction from 750 g. starch	Molecular weight by viscosity in chloral hydrate	Molecular weight by osmotic pressure of acetate
I	1.8	13,000	60,000
II	1.0	23,000	
III	10.1	26,000	
IV	12.5	35,000	

Meyer (62) fractionates amylopectin by treating the 94% (to 92%) of corn starch insoluble in the water extraction at 70° C. with successive portions of 33% chloral hydrate at pH 7.0, using increasing temperatures for each treatment. These solutions are treated with acetone to precipitate the solids. The results of this fractionation are shown in Table IX. Fraction I is claimed to be amylose and Fraction II is a mixture of amylose and amylopectin, according to Meyer.

The inadequacy of such data to support the views given as to the composition of starch and the structure of its components is made evident by comparison of the above results with fractionation procedures given by Kerr and coworkers, and by a comparison of the description of the properties of the various constituents by the two groups of workers, respectively. In the latter connection it is to be noted that Meyer states³ that amylopectin separated from a water solution gives the same crystalline interferences as amylose; that amylopectin rapidly loses its solubility in water in the course of drying; that its aqueous solutions become turbid after a few hours, are more turbid than those of amylose, and deposit quantitatively in a few days; and that amylopectin exhibits a

³ See particularly reference (72).

TABLE IX

Yields and Properties of Corn Amylopectin, According to K. Meyer

Fraction No.	Temperature of extraction	Total starch extracted	$\frac{\eta_{sp}}{C}$ * ($C = 1$)	Molecular weight
	$^{\circ}C.$	<i>per cent</i>		
I	70 (H ₂ O)	0- 8	0.51	50,000
II	20-25 ¹ ₂	8-12	0.60	60,000
III	35-40	12-17	0.73	73,000
IV	45-50 ¹ ₄	17-25	1.31	131,000
V	55	25-35	2.25	225,000
VI	60-62	35-69	Insoluble	
VII	75	69-95	"	
VIII	80	95-99.4	"	

* Acetates in hydrazine hydrate, in which they saponify.

higher solution viscosity than amylose (see Table IX).⁴ It should be noted, however, that in a recent summary (71) Meyer concludes that it is impossible to separate components of the amylose and amylopectin types quantitatively. This conclusion agrees with that of Kerr and coworkers in respect to methods of extraction with hot water which will be reviewed after a discussion of a method for the crystallization of constituents of the amylose type.

The laboratories of the latter investigators have made several contributions to a clarification of the problem of starch composition. Schoch (22) made the novel observation that certain higher alcohols, for example butanol, are much more selective in precipitating a fraction of starch of the amylose type than the lower alcohols previously used. It was found, after starch was completely solubilized by autoclaving, that, by the addition of as little as the 8 to 10% of butanol required to saturate the solution, approximately 22% of the starch solids had been consistently precipitated. The precipitate is separated in the supercentrifuge and appears to be composed of spheroids which are semicrystalline in appearance (22). This product is reported to be quite different in properties from the 78% of starch solids which will not precipitate with the added butanol but which are recovered by the addition of substantial quantities of a more soluble alcohol. This more soluble fraction, when dried, is readily soluble in water and gives a clear stable solution. As discussed elsewhere in this text, the latter fraction gives an amorphous x-ray diffraction pattern. The butanol-precipitable fraction is, as first recovered, soluble in hot water, but rapidly retrogrades to an insoluble product. When the butanol-precipitated fraction is dried in the oven or air-dried, it is completely insoluble in water (73). Later, Bates, French, and Rundle (74) supported the view that a fairly complete separation of linear and branched configurations is obtained by such a procedure. By a novel method of iodine titration and by the gravimetric procedure for

⁴ Dilute solutions in hydrazine hydrate.

butanol precipitation, a variety of starches has been analyzed and the yields of total amylose and amylopectin compared. These results show variations in amylose content from zero for waxy maize up to 35% for lily bulb starch. The existence of only two molecular patterns is postulated. A pure, crystalline, corn amylose as prepared by Kerr and Severson is used for standardization in the technique for iodine titration.

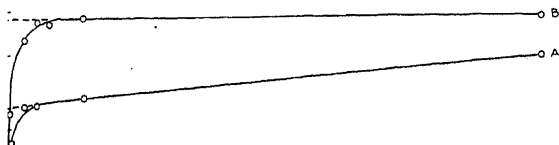


FIG. 50. Estimation of conversion limits to maltose of (Curve A) corn starch, and (Curve B) its crystalline amylose by β -amylase. The abscissa represents hours of conversion; the ordinate, \log_{10} (per cent converted/10).

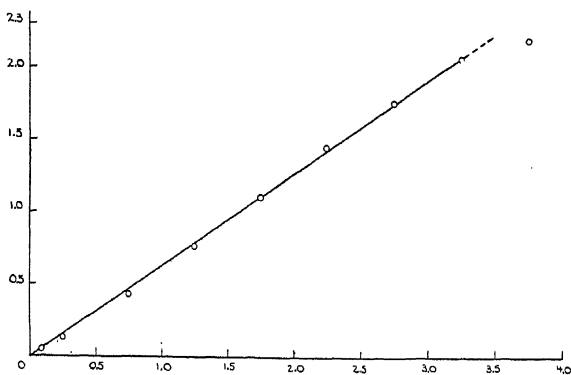


FIG. 51. Rate of conversion of corn, crystalline amylose to maltose by β -amylase. The abscissa represents hours of conversion; the ordinate, $\log_{10} (1/(1-x))$.

Corn and potato starches show about the same distribution by both methods of analysis, 22% linear and 78% branched. For tapioca, there may be some discrepancy in the results obtained by the two methods. Only 17% of linear constituents is reported by iodine titration but when the same technique of butanol precipitation is applied to tapioca starch as has been applied to corn and potato starches, about 21% is precipitated.⁵

⁵ Unpublished data. See also Chapter XVII for revised values for the amylose content of corn, potato, and tapioca starches.

Kerr and Severson (66, 21) applied a butanol precipitation to a hot water extract of corn and potato starches and succeeded in crystallizing out the major component. After several recrystallizations from water and butanol a starch component of the amylose type was finally isolated from the starches. The corn, crystalline amylose is reported to be essentially a linear chain of 1-4 α -glucoside-linked glucopyranose units.

Fig. 50 illustrates the method of obtaining the limit of conversion of the amylose to maltose by β -amylase and compares the results of the same technique as applied to whole corn starch. A carbohydrate concentration of 4 g. per liter is used at 47° C. and pH 6.0 in the conversion. A small β -amylase addition is employed. The limit of conversion is about 93% to reducing sugars, estimated as maltose by reduction with alkaline ferrieyanide. In Fig. 51 the rate of hydrolysis to maltose is plotted as a first order reaction. A small enzyme concentration was used to reduce the reaction rate for greater accuracy in calculation. The reason the reaction does not proceed to completion at its original rate is to be ascribed to the associative tendencies and colloidal instability of the amylose even at high dilution, which effects become visible in the later stages of the reaction by the appearance of a haze. It is also quite possible that a perfectly linear component would not be 100% hydrolyzed by the conditions used because of a necessary minimum chain length required for the enzyme to approach in order to effect a speedy scission. However, in this case, the longer the chain originally, the more closely the conversion limit should appear to approach 100%. The unsupported and unqualified statement that a fraction of starch is converted completely to maltose is rather meaningless and of little theoretical significance. Rather crude fractions will become 100% converted if enzyme is added in large excess or if the time of contact with active enzyme is of sufficient duration.

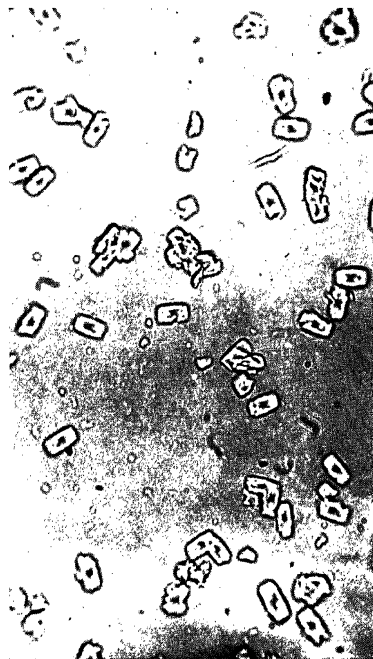


FIG. 52. Corn, crystalline amylose ($\times 300$)

Support for the structure ascribed and confirmation for the crystallinity and purity of the corn, crystalline amylose are given in the work of Bates, French, and Rundle (74) and of Rundle and French (75). Fig. 52 is an illustration of the platelet-shaped crystals of this originally warm water-soluble corn amylose. It is apparently composed of helically shaped coils of linear molecules, the axes

of which coils are perpendicular to the plane of the largest face of the crystal. Fig. 53 shows the x-ray diffraction pattern obtained with the crystalline amylose. This may be compared with Fig. 54 which shows the pattern obtained with the purified (21) more alcohol-soluble fraction and is characteristic of amorphous materials.

A most important and engaging question is, how does this amylose differ from that balance of the corn starch, which under certain circumstances, at

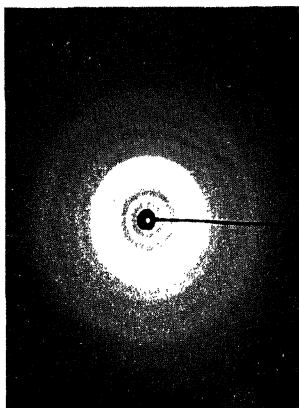


FIG. 53. X-ray diffraction pattern of corn, crystalline amylose.

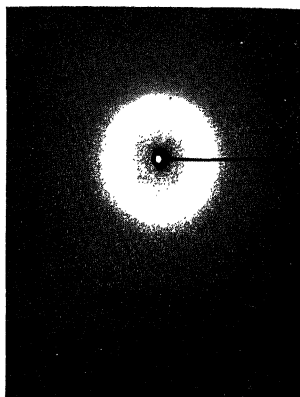


FIG. 54. X-ray diffraction pattern of a purified branched fraction of corn starch.

least, behaves as though it also is linear in structure? This balance is made up of two major divisions, a portion of the starch which becomes soluble in water at temperatures higher than those used to extract the first crystalline product described and a portion of the starch which is relatively insoluble even in boiling water but which is gradually solubilized in the autoclave at 120° C. All three divisions of material make up the total of 22% of corn starch which is precipitable with butanol after being autoclaved.

According to Meyer, we might expect that these divisions represent different orders of chain lengths; those that are most soluble are the shortest, and those that are least soluble the longest. This explanation becomes inadequate, however, when, in pursuing the theory that all amylose fractions are unbranched, the crystallizable, warm water-soluble amyloses of potato and tapioca starches are examined and compared with that for corn starch. Crystalline amylose from potato has been described by Kerr and Severson (66). Its limit of conversion to maltose by β -amylase is 97%. Crystalline amylose from tapioca is prepared by similar procedures. Figs. 55 and 56 show the more elongated or needle-like crystals obtained from these two starches. Both are at least as soluble as the most soluble corn amylose, are patently of greater molecular

harmonize with the fact, noted many times by the author, that, when solutions of crystalline amyloses from corn, potato, and tapioca starches are treated with β -amylase in dilute solution, retrogradation effects are noted during the course of the hydrolysis only in the case of the crystalline amylose from corn. If the attack of the β -amylase is an orderly removal of maltose units, as generally presumed (37), then it might be expected that at some stage during the hydrolysis the amylose chains of potato and tapioca starches would pass through the chain length zone which is optimal for orientation.

The suspicion is therefore created that even the simplest molecular structures in potato and tapioca starches are not perfectly linear, but that a limited amount of branching exists. The configuration might be represented by a V, or fork-shaped unit, with the point of branching quite close to the terminal aldose group. When these amyloses are dissolved in water, even a small amount of intermolecular bonding of the side arms of a V, possibly through hydrogen bonds from hydroxyl to water to hydroxyl, would produce a highly reticulated pattern, the colloidal stability of which should be definitely increased over a simple linear chain. It is believed significant that, in contrast to the action of β -amylase, a mild hydrolytic action by hydrogen ions on potato starch (76, 77) or the potato amylose, even a prolonged boiling in water, causes the potato starch paste to congeal rapidly on cooling and the potato amylose to become more unstable in solution. In the case of the amylose, at least, the author has noted that the change is irreversible, since the retrograded product after solution in alkali behaves very similarly to corn amylose when the solution is neutralized. That part of the amylose fraction of both potato and tapioca starch which becomes water-soluble only at higher temperatures is, quite probably, more complicated in structure than the crystalline amyloses.

Therefore, it seems very logical to believe that the solubility of all of these components is primarily dependent upon the manner in which they have become entangled within the starch. This condition is related to the complexity of their structures, the extent to which these structures have become associated through cross-bonding, and, possibly, the manner in which their configurations have been altered in the course of association not only with carbohydrate residues but with other material; *e.g.*, the fatty acids.

The possibilities of hot water extraction of starch to separate and determine the differences in the various fractions of the "linear" components of corn starch have been investigated by the writer.⁶ Native corn starch partially defatted and extensively defatted (78, 79) were extracted with water for 1 hr., with gentle stirring, at various temperatures, under a reflux. The pH in all instances was between 6.0 and 6.5. 2.5 g. of starch per 100 cc. of water were used, and in the case of defatted starch, a dilution of 0.5 g. per 100 cc. was used as well to determine whether simple solubility phenomena were being studied. After the extractions, the liquors were filtered by gravity, while hot, and crystal-clear

⁶ Unpublished data.

filtrates were obtained. An aliquot of each filtrate was examined to determine (a) the total solids solubilized, (b) the β -amylase conversion limit to maltose of the extracted solids, (c) the per cent of solids crystallizable by saturating a 1% solution of the extracted solids with butanol, (d) the β -amylase conversion limit of the crystals isolated, and (e) the maximum yield of crystallizable material obtainable, assuming complete recovery of the liquid phase. In all cases it is assumed that the concentration of the extracted substance is about the same in the liquid obtained as filtrate and in the liquid left with the residual starch.

TABLE X
Hot Water Extraction of Corn Starches

Starch; fat content *	Starch per 100 cc. water	Extraction tempera- ture	Total starch solubilized	C. L.† of total solids extracted	Solids extracted pptd. with butanol	Estimated maximum yield of crystals	C. L.† of crystals
<i>per cent</i>	<i>g.</i>	<i>° C.</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	
Native, 0.920	2.5	70	6.00	75.2	80.0	4.8	93
Defatted, 0.252	2.5	70	9.88	82.4	89.4	8.83	91.5
	2.5	75	12.05	88.7	89.1	10.74	93.0
	2.5	80	15.64	86.1	87.5	13.68	91.3
	2.5	85	16.90	85.1	86.1	14.55	90.5
	2.5	90	19.25	80.7	85.1	16.38	90.2
	2.5	125	100	63	22	22	81-83
	0.5	70	11.12	76	84.5	9.40	89.6
Defatted, 0.190	2.5	70	14.82	84.5	84.8	12.57	89.1
	0.5	70	15.80	80.7	84.3	13.31	77.8

* See reference (79).

† See the text for the conversion limit (C. L.) to maltose by β -amylase.

The results, given in Table X, show that only about 6% of native corn starch is solubilized at 70° C. by such a procedure as the one described. This value is of the same order as that reported by Meyer. The product is, as might be expected, of rather low purity in respect to "amylose," as previously reported by Kerr, Trubell, and Severson (20). The impurities are such, however, that on addition of butanol a product of relatively high purity crystallizes out.

As corn starch is defatted, the solution of larger amounts of starch solids by hot water is effected. But it would appear that it becomes more difficult by adding butanol to obtain crystals which are converted to as high a limit by β -amylase.

The effect of increasing the temperature of extraction is shown for the partially defatted starch. Higher temperatures again result in larger quantities of solubilized material. The purity of the extract, in respect to "amylose," reaches a maximum at about 75° C., and then falls off with increasing temperature. In spite of the fact that larger quantities of crystallizable material are obtained at higher temperatures until complete solubility of the starch is reached at 125° C.,

the β -amylase conversion limit of these crystals falls off after a peak is reached in extraction at 75° C. From these experiments it may be noted that, to secure the largest yields of highly convertible crystals, one should employ a partially defatted starch, rather than one extensively pretreated with boiling methanol-water solutions and employ 70–75° C. for the water extraction.

The effect of increasing dilution in the extraction of the starch is shown both for partially and extensively defatted corn starch. These experiments, together with those previously reported (66) in which native corn starch is treated in several successive extractions at the same temperature, indicate that the yield of soluble material obtained under a given set of conditions is not governed solely by a solubility equilibrium in respect to the total "linear" fraction of corn starch. Rather, there is a more or less defined proportion which dissolves in the extraction medium at a given temperature. This is more strikingly demonstrated by using defatted corn starch for extraction, the failure of the use of which in separation procedures may lead to an erroneous impression concerning the composition of corn starch (79). For the third starch listed in Table X the results of three successive extractions at 70° C., pH 6.2, and at a dilution of 2.5% are 15.05%, 1.10%, and 0.28% solubilized, respectively.

The above data may be interpreted by assuming a variable degree of entanglement of the components of the granule, for if excessive stresses are applied to these granules which would tend to break them down, as in passing the extraction mixture at 70° C. through a supercentrifuge at 40,000 R.P.M., as high as 50 to 75% of the total weight of the granule will appear to have been solubilized in the clarified centrifuge.

The results reviewed suggest that a material difference exists between fractions of starch which are solubilized at lower temperatures and those solubilized at higher temperatures in water. More particularly, it would appear that the character of the linear polymer, or butanol-precipitable constituents in these extracts, varies as the extraction temperature is increased. Further information on the nature of these differences is obtained from the work of Kerr and coworkers (80). Fractions of the butanol-precipitable components of corn starch were progressively separated as follows. Partially defatted corn starch (500 g. of the second starch listed in Table X) was extracted with water at 75° C. according to the above given procedures. After filtration of the extract by gravity, overnight under toluene, the insoluble residue was collected and extracted in a similar fashion at 90° C. After a second filtration, the remaining insoluble residue was solubilized in 6 liters of 0.67 *N* NaOH at room temperature. The solution was diluted to 20 liters during the course of neutralization. HCl was used to adjust the acidity to pH 6.0. After heating to 70° C. the solution was saturated with butanol and slowly cooled to room temperature over a period of 72 hrs. The precipitated solids were recovered by passing the liquors through a supercentrifuge at 40,000 R.P.M. The two hot water extracts were separately concentrated by vacuum distillation to solutions which contained approximately 1% of solids, were warmed to 80° C., saturated with butanol, and allowed to cool

slowly over a period of 48 hrs. to room temperature. The precipitates were collected by centrifuging at 2000 R.P.M. All three precipitates were separately washed with ice water which had been saturated with butanol and then crystallized twice more by redissolving in hot water, filtering and saturating the hot filtrate with butanol. The three batches of crystals were repeatedly washed with ice water saturated with butanol and were dehydrated by four successive washes in absolute methanol followed by prolonged vacuum drying at room temperature over sulfuric acid.

Table XI summarizes the data on the fractionation.

TABLE XI
*Fractionation of Partially Defatted Corn Starch **
(500 grams)

Grams, solids	Water extraction at 75° C.	Water extraction at 90° C.	Water-insoluble at 90° C.
Extracted	54.570	23.020	
In first mother liquor . . .	5.611	4.058	
In first wash	0.5846	0.4165	18.260
In second mother liquor	3.369	0.2358	5.762
In third mother liquor .	2.492	1.051	2.925
In final wash of crystals	0.012	0.066	0.063
Yield of crystals	42.2	14.1	23.7

* Using butanol to crystallize the amyloses from a water extraction or solution of starch.

The solution viscosities of the three linear polymer fractions of corn starch were determined in ethylenediamine at 25° C. in an Ostwald viscosimeter and compared against those of three subfractions ⁷ of the branched polymer fraction. For further comparison the viscosities of the crystalline amyloses of potato

⁷ The subfractionation of the butanol non-precipitable fraction of starch may be accomplished by adding progressively larger amounts of butanol and methanol to a hot water solution of the fraction and allowing the solution to cool slowly in a closed vessel. A subfractionation is obtained by adding 20 cc. of butanol to a hot 3% solution of the amylopectin, under a reflux, and then adding dropwise, with vigorous stirring, 18 cc. of methanol, which solubilizes the excess butanol. The fine grain material which separates on cooling may be centrifuged and "recrystallized" from a hot solution which contains the same proportions of water, butanol, and methanol that are used in the first precipitation. The product studied had a β -amylase conversion limit of 58.7% as first precipitated and 60.9% and 59.6% respectively after two recrystallizations.

A second subfraction, with a β -amylase conversion limit of 55%, precipitates when the amount of butanol is increased to 24.25 cc. and the methanol to 20 cc. for each 100 cc. of the water solution of the amylopectin originally used. The product studied was additionally treated in water solution with three successive lots of cotton (47 g. of cotton for each 10 g. of fraction in 1 liter of solution) to insure the removal of the last traces of linear polymer constituents.

The third subfraction, practically the entire balance of dry solids remaining in solution, precipitates as a gummy mass when a large excess of methanol is added. It represents about 10 to 15% of the parent corn starch, stains a clear wine-red color with iodine, and has a β -amylase conversion limit of 50 to 52%.

and tapioca starches, previously described, and of glycogen were also determined. These results were interpreted according to the suggestion of Huggins (81) who has shown that a linear relationship exists between specific viscosity divided by concentration (η_{sp}/C) and specific viscosity (η_{sp}). This relationship has been experimentally confirmed for starch fractions by Foster and Hixon (67). Huggins has shown that the ratio of the slope of the straight line obtained to its intercept depends on the type of solute and solvent and on the temperature but not (or very little) on the molecular weight of the solute. In characterizing a particular solute-solvent system in this manner, a factor is obtained which is related to the way in which the molecules of the solvent and the submolecules of the dis-

TABLE XII
Characteristics of Starch Fractions

Starch fraction	Intrinsic viscosity	Conversion limit *	Alkali labile number †	Iodine titration, "linear" material ‡ per cent
<i>Corn starch</i>				
<i>Amylopectin</i>				
Subfraction I	1.445	60	3.2	11.8
" II	0.846	55	11.3	8.35
" III	0.526	50-52	13.7	0.00
<i>Butanol precipitate</i>				
Of 75° C. soluble ext.	0.660	93	35	100.0
" 90° C. " "	1.172		34	100.0
From 90° C. ins. residue	1.158		23	94.0
<i>Tapioca crystalline amylose</i>	1.006	89	17	100.0
<i>Potato crystalline amylose</i>	1.125	97	21	100.0
<i>(Glycogen)</i>	0.099			0.0

* Per cent conversion to maltose by β -amylase. See the text.

† See reference (58).

‡ Potentiometric titration with iodine. See the text.

solved polymer interact with each other during the flow of the liquid around and through the solute particles. The data of many workers show that the deduction of Huggins is substantially correct. Therefore, if differences are noted for a series of related polymers when their solution viscosities are determined under fixed experimental conditions and are compared in the manner suggested, it may be concluded that the polymers are of different shape, and in this sense they must be considered as being different systems.

That intrinsic viscosity, $[\eta]$, is a function of the shape of a solute in a given solvent is now a generally accepted fact. In the comparison of an homologous series, intrinsic viscosity is related to molecular weight or "chain length," and, in the comparison of similar polymers, intrinsic viscosity is related to the axial ratio of the solute. It should be noted, however, that since the result obtained

is a weight-average molecular weight, the value is materially affected by the presence of small amounts of impurities which have a greater "chain length," but practically not at all by small amounts of impurities of lesser "chain length." Extrapolation of the linear function, $\eta_{sp}/C \propto \eta_{sp}$, to its intercept on the η_{sp}/C axis affords a convenient and accurate means of obtaining the intrinsic viscosity.

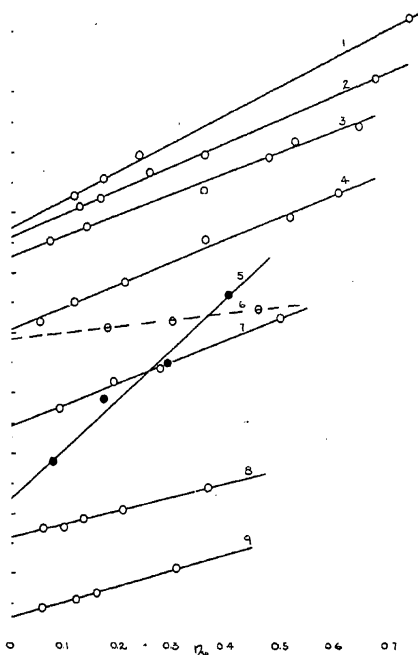


Fig. 57. Viscosities of solutions of starch subfractions and glycogen in ethylenediamine. Curve 1, butanol precipitate of fraction of corn starch water-soluble at 90° C.; Curve 2, butanol precipitate of fraction of corn starch water-insoluble at 90° C.; Curve 3, potato, crystalline amylose; Curve 4, tapioca, crystalline amylose; Curve 5, corn amylopectin subfraction I (both η_{sp}/C and η_{sp} are divided by 2); Curve 6, glycogen (η_{sp}/C multiplied by 10); Curve 7, corn amylopectin subfraction II; Curve 8, butanol precipitate of fraction of corn starch water-soluble at 75° C.; Curve 9, corn amylopectin subfraction III.

An inspection of the viscometric data for starch fractions, given graphically in Fig. 57, shows that the subfractions of both the linear and branched polymer fractions of corn starch give curves with various slopes. Therefore, neither major fraction is homogeneous in respect to the structure of its respective components. Since the reported data for the behavior of the linear fraction soluble in water at 75° C. show that the structure of these molecules is unbranched, a different shape, particularly a definitely greater axial ratio, must be assumed for the fractions soluble in water at higher temperatures. The rate and completeness with which the 75° C. soluble fraction spontaneously precipitates, or retrogrades,

from a water solution do not support the view that a smaller axial ratio is the result of a physical variation in shape, *e.g.* a linear chain coiled to form a helical spiral, since retrogradation presumably results from cross-bonding of parallelly oriented linear chains. The 90° C. soluble fraction is materially more stable in aqueous solution. The data presented in Table XII, particularly the "linear content" by iodine titration,⁸ are not in accord with the view that the linear polymer fractions of corn starch which are insoluble in water at 75° C. (especially the 90° C. soluble fraction) are contaminated to any significant extent with the branched polymer fraction. Rather, it seems more reasonable to conclude that the structure of these linear components is branched to some extent and that long unbranched sections or members exist in these branched structures.

The low intrinsic viscosity obtained for the end member of the corn amylopectin fraction, subfraction III, is indicative of a less asymmetric molecule than exists in any other subfraction of corn starch. The very low conversion limit to maltose with β -amylase and the result of the analysis for linear material by iodine titration, which is zero for this fraction, show that its structure is highly branched or ramified and that the terminal branches of the complex structure are relatively short. The alkali labile value (58) does not support the view that this fraction consists of short linear chains, too short to precipitate with butanol in the primary fractionation, too short to orient with iodine molecules, and of insufficient length to be acted upon by β -amylase. Alkali labile numbers vary inversely with the chain length of an unbranched, 1-4 α -glucosidically linked glucopyranose polymer and with the complexity of structure such as the number of side branches since the alkaline degradation involved in the test starts from the terminal aldose group, proceeds by enediol splitting and not by direct hydrolytic scission and since the time of reaction is limited in the test. If amylopectin subfraction III were composed of short linear chains, the alkali labile number would be larger than the value found for the unbranched fraction rather than very much lower.

The axial ratios of the components of subfractions I and II are very much greater than those of subfraction III. Indeed, the intrinsic viscosity of subfraction I indicates that its structure is more extended than that of any of the so called linear polymer fractions, and, in this sense, it is more linear than the unbranched fraction of corn starch. The iodine titration values and the limits of conversion to maltose with β -amylase indicate the presence of long unbranched members in the structures of the molecules of subfractions I and II. The anomalous viscosity of these two subfractions cannot logically be attributed to the presence of minor amounts of impurities consisting of unbranched molecules for reasons already discussed, and a comparison of the alkali labile numbers for these subfractions, particularly the extremely low value found for subfraction I, leads to a similar conclusion, that the subfractions cannot be considered simply as a mixture of unbranched molecules and of branched structures such as are present

⁸ See Chapter XVII.

in amylopectin subfraction III. An alkali labile value of 3 cannot be obtained as an average of values of the order of 35 and 14. Nevertheless, it is to be noted that a weighted average of the alkali labile numbers for the three amylopectin subfractions is 6.3 which is in fair agreement with the value of 5.6 found by experiment for the whole fraction.

A comparison of the solution viscosity of glycogen in ethylenediamine shows the results to be expected from a structure which contains many short branches and which is so highly ramified that, notwithstanding its very large molecular weight, very low viscosities are obtained. Obviously, the ramified structure proposed by Meyer (52) for amylopectin, which should also result in a compact and non-elongated shape, is not consistent with the results obtained by Kerr and coworkers for any of the subfractions of corn amylopectin. Rather, the data reviewed suggest that all of the components of corn starch are relatively asymmetric in shape and that they vary from relatively short, unbranched structures to those which contain many short branches. Intermediate to these two types are molecules which contain comparatively long sections or members which are unbranched and which may contribute to the formation of a highly elongated molecular structure.

An examination of the data of Kerr and coworkers for the least complexly constituted components of potato and tapioca starches, the crystalline amyloses previously mentioned, does not lend support to the view that these starches are composed simply of a mixture of unbranched chains and molecules which are branched according to one definite pattern. Although it is evident that the axial ratio of both amyloses is greater than that of the unbranched corn fraction, the slopes of their curves, plotting η_{sp}/C against η_{sp} , suggest a variation in structure as a further difference between the amyloses. Also, the greater colloidal stability of the potato amylose, and particularly of the tapioca amylose, supports the view that some branching exists in their structures. If the assumption is made that the only difference between the three amyloses is one of chain length, then it follows that a length of chain exists (such as is found in the corn amylose) which is optimal for colloidal instability or retrogradation, and the relative stability of tapioca amylose should be less than that of potato. However, the reverse is true. A similar conclusion follows from an inspection of the alkali labile numbers for these two amyloses; the tapioca amylose should have the greater axial ratio or chain length, which it does not. Apparently, some branching exists in the structure of these two amyloses, and the presence of long linear members in the structure is indicated by the various data presented. To explain the high limit of conversion of the potato amylose, it may be assumed that an anomaly in the structure does not permanently block the activity of the enzyme but reduces the rate of its action, and that when only one or a very limited number of branched points are present in a comparatively large molecule, the rate is imperceptibly reduced and the limit of conversion is not materially affected owing to the time permitted for the reaction to take place.

It is obvious that the views presented rest on the validity of several assumptions. Two, at least, are worthy of further consideration. It has been assumed that the various subfractions of starch studied are relatively homogeneous, or, at least, are free from components which vary materially in size or shape. It has also been assumed that the anomalous viscosities reported are due to the more common and simple interactions between the solute molecules of high polymers and between solute and solvent. Although it would appear unlikely that at the higher dilutions used in the study of starch polymers in ethylenediamine, the critical concentration range for gelation effects had been exceeded, this effect cannot be ignored, nor can other possible effects, such as the possible interaction between symmetrical but burr-like structures, concerning which little is known. The results of experiments in progress in several laboratories on the osmotic pressure, sedimentation velocity, and exhaustive methylation of these and similar preparations of starch fractions are very much needed in order to clarify completely the remaining questions relating to the structure of the starch molecules.

4. Non-carbohydrate Constituents of Starch. Whether or not starch is pure carbohydrate has been the subject of extensive research. It has been proposed that, in addition to the glucose residues of which the molecules of starch are composed, there are other substances combined with them. Various theories have been developed from this assumption to explain certain anomalies in the chemical and physical behavior of the starches.

It has been proved by analysis that, when various starches are purified by non-hydrolytic agents such as water at low temperatures and ether and then are

TABLE XIII

*Some Non-carbohydrate Constituents of Certain Starches**

The values are given in per cent.

	Potato	Wheat	Corn	Rice	Sago	Tapioca	Arrowroot
P ₂ O ₅	0.176	0.149	0.045	0.015		0.017	
" †	0.287	0.265	0.069	0.243	0.054	0.062	0.023
SiO ₂	0.069	0.019					
SO ₃	0.008	0.066					
CaO	0.058	0.042	0.024	0.014	0.025	0.015	0.112
MgO	0.001	0.026	0.014	0.040	0.005	0.005	0.003
K ₂ O	0.018	0.027					
" †	0.072	0.087	0.028	0.063	0.082	0.024	0.028
Na ₂ O	0.008	0.032	0.041	0.283	0.032	0.027	0.020
Fe ₂ O ₃	Trace	Trace					
N	0.0113	0.0488					
Fat ‡	0.04		0.61	0.83	0.11	0.12	

* The table is compiled principally from data from the works of Samec and Blinc and of Taylor, referred to in the text, and from the report of Edwards and Ripperton (82).

† Edwards and Ripperton used native starch for the analysis.

‡ Difference between total fat and ether-soluble extract.

completely hydrolyzed by weak hydrochloric acid, appreciable amounts of phosphates, fatty acids, nitrogenous bodies, silica, and other substances may be recovered. Table XIII, taken principally from summaries given by Samec and Blinc (83), gives analyses of some of the more important starches. Both potato and wheat starch yield considerable amounts of phosphate; corn yields fatty acids, amino acids, and occasionally silica; rice and wheat yield nitrogenous material, and so forth. Inasmuch as all of these substances possess acidic functions (the amino acids, basic functions as well), it is not an illogical assumption that some of the many hydroxyl groups present in the fundamental starch molecule may be esterified with the acids. If such is the case, then molecules esterified with phosphoric acid might be expected to be more lyophobic, and an esterified glucopyranose unit might be expected to present a barrier to the systematic and highly specific activity of enzymes. Molecules esterified with fatty acids might be expected to be more soluble in alkaline solution, but tend to become insoluble, or be originally insoluble, in neutral or acid solution. Combined silicic acid might be expected to confer a measure of insolubility on starch fractions or a tendency to form gels in neutral or acid media, which physical states would tend to resist enzymic attack. The literature on this phase of starch chemistry is very extensive, not only in experimental evidence to support the theories given but also on the alternative side of the question. Inasmuch as many of these questions have been definitely settled in recent years to the effect that with only a very limited number of exceptions starch is now known to be pure carbohydrate, the subject has lost much of its former importance. Hence only a brief reference will be made to sources in which the theories and data pertaining to this discussion have been more completely treated.

Samec (84) has discussed the rôle of phosphate in potato starch. The early work of Northrop and Nelson (85) showed that, by progressive hydrolysis of the starch to products of very low molecular magnitude, fractions could be separated from the hydrolysate which had progressively higher phosphorus contents. Following the experiments of Maquenne and the procedures used in more recent years by Tanret, Baldwin, and Meyer to demonstrate the non-homogeneity of starch, the early work of Sherman and Baker (86) indicated that the phosphate was unevenly distributed in potato starch in that a hot water extract of the latter contained 0.023% P_2O_5 and the swollen residue 0.147%. The result was confirmed by Baldwin (19). Kerr (66) observed a very unequal distribution of phosphorus between the portions of potato starch that are more soluble in alcohol-water solutions and those that are less so but not in the case of corn starch. More recently Kerr⁹ has found no phosphorus in the crystals of amylose isolated by means of butanol from hot water extracts of potato starch. Samec showed that if potato starch sols are electrolyzed there is an uneven distribution in phosphorus between the portion which migrates and the portion which does not. He therefore ascribed the ability of the former fraction to move to the positive

⁹ Unpublished data.

electrode to the esterified phosphoric acid group which is ionized to an anion and confers a negative charge on the carbohydrate. Since this fraction of potato starch is essentially amylopectin in character, Samec proposed that the essential difference between amylose and amylopectin was explainable on the basis of esterified phosphate. Gruzewska (87) had noted that preparations described as potato amylopectin migrated to the anode in an electrolysis cell. Amylose preparations were phosphorylated and were claimed to yield viscous solutions comparable to natural amylopectin in many respects. Myrbäck (38) suggested that the reason certain fractions of starch could not be degraded completely by β -amylase was because of an irregularity in constitution such as might be caused by the presence of a glucopyranose unit esterified with phosphate. Samec extended his amylophosphoric acid theory for amylopectin to wheat and to other starches. However, whereas Posternak (88) was able to extend the results of Northrop and Nelson and recover glucose-6-phosphoric ester from the end-products of a potato starch degradation, thus proving that some phosphorus is combined with potato starch, this investigator was not able to obtain similar results with wheat starch. By enzymic degradation, Posternak obtains a tetrose phosphate as the end-product which after acid hydrolysis yields glucose-6-phosphate, identical to the sugar isolated by Robison and King (89) from the end-products of starch fermentations.

Wheat starch, as well as corn starch, yields as a final product α - and β -glycerophosphate. From the fact that both the phosphorus- and nitrogen-containing bodies may be removed by extracting gelatinized wheat starch with alcoholic solutions, although potato starch so treated is not materially reduced in respect to its phosphorus content, Posternak concludes that the phosphorus in wheat starch is present as a phospholipid and as such is present as an impurity or as a simple physical mixture. Samec and Blinc point out as significant that the intact wheat starch granules are not materially reduced in phosphorus content by simple Soxhlet extraction with alcohol, and that best results are obtained after treatment of the granules with alkali, particularly by an alkaline gelatinization of the starch. However, quite recently Schoch (78) has shown that Soxhlet extraction of whole wheat starch for 48 hrs. with 80% dioxane reduces the phosphorus content to the vanishing point (from 0.054% to 0.008%), whereas the phosphorus in potato starch only drops from 0.095% to 0.087% in a similar treatment. It is evident, therefore, that phosphate can exist in chemical union with some starch molecules, in particular potato amylopectin and possibly amylopectins from other root starches such as arrowroot. The behavior of amylopectins in general, however, cannot be attributed to esterified phosphate. Rather their distinguishing character as a class is dependent on more fundamental variations in structure, as previously discussed. The subject reviewed is a classical example of overextending to other starches a hypothesis formed from an intensive study of only one particular starch.

It is interesting to note from work presented to date that when phosphate is found esterified with starch in nature one would conclude that the union is

through the carbon atom 6 of the glucopyranose units. Yet when synthetic glucopyranose chains are formed from glucose-1-phosphate (Cori ester) by the action of phosphorylases, the phosphate group remains, if at all, either on carbon atom 1 or 4. If the latter system of reactions represents the synthesis of starch units in nature, it is difficult to conceive how the phosphate remains in the molecules in some starches and not in others, and in the former case how it is rearranged to an ester linkage on carbon atom 6 unless one assumes the presence of a phosphoglucomutase similar to the enzyme separated by Cori and coworkers. This enzyme causes glucose-1-phosphate to assume the more stable form, glucose-6-phosphate which enzyme is found in plants, yeast, and other tissues. Perhaps the subject should again be reviewed in the light of our newer knowledge concerning carbohydrate synthesis, which will be discussed in a following section.

It has been known for many years that some starches contain fatty material in such close association that they cannot be purified from these constituents by mechanical or physical means in starch manufacture. Starch factories which convert starch with acid to produce sirups and sugars must remove considerable quantities of an insoluble "refinery residue" in the clarification of these fluid hydrolysates. In the use of corn and some other starches, this residue contains substantial amounts of fatty acids. More than 50 yrs. ago, Sostegni (90) hydrolyzed rice starch and showed that the insoluble residues remaining contained a fatty material, soluble in ether, which melted at about 48° C.

Taylor and Nelson (91) and Taylor and his students (92-96) have made an extensive study of the fatty material associated with corn starch. After the starch was thoroughly purified and extracted with ether, it yielded about 0.6% of fatty material after hydrolysis, and in the residue palmitic, oleic, and linolic acids have been identified. Lehrman (97) found the same acids after a similar treatment of tapioca starch, and in addition, linolenic acid. Lehrman and Kabat (98) could find no fatty acids associated with potato starch, however.

Taylor and his students attempted to show that these fatty acids were esterified with a portion of the starch, which they called α -amylose, and that the properties of this component, such as its anodic migration in an electrolysis cell, its solution in alkali, and precipitation therefrom on acidification, depended on the presence of esterified fatty acid. It was reasoned that the original insolubility of this component in corn starch was explained and the complex was, accordingly, differentiated from retrograded amylose. According to the conception of these workers, the balance of starch which remains after removal of α -amylose, which they named β -amylose, corresponds to amylose. The term α -amylose was used synonymously with amylopectin. β -Amylose when retrograded, in the sense that it has become insoluble in water, does not reprecipitate when dissolved in alkali and the solution is acidified.

Kerr and coworkers examined the critical experiments presented (24) but finally concluded that β -amylose does not correspond to amylose (20). It would now appear that α -amylose represents a fraction of the linear polymer fraction of corn starch, and β -amylose the rest of the linear polymers mixed with practically

all of the more complexly constituted components. Rask and Phelps (99) showed that the fatty acids from corn starch are quantitatively extracted by alcoholic ammonia. Taylor and Werntz (93) reported that it required eight successive treatments with this solvent to reduce the fatty acid content to a negligible value. The suspicion arises therefore that a gradual saponification of the acids resulted from such treatment. The question was finally settled by Schoch (100, 78) who showed that neutral, water-miscible solvents, such as alcohol and dioxane, are sufficient for extraction of the fat from corn, wheat, and rice starches. Lehrman (101) finally concluded that the fatty acids present in starch are adsorbed.

Clayson and Schryver (102), Schryver and Thomas (103), Ling and Nanji (104), and others have observed that some starches, particularly the cereal starches, appear to contain a component resistant to the action of ungerminated barley diastase. The greater resistance of these starches, compared to tapioca and potato starches for example, would accordingly be explained. Ling and Nanji analyzed this fraction and consistently found from 0.83 to 0.98% of SiO_2 , from which they concluded that the product, called amylohemiacellulose, is a salt of a silicic acid ester of amylose.

However, Samec (105) found that a dilute solution of hydrofluoric acid removes the silica from starch and that the purified starch yields the same percentage of amylohemiacellulose and is just as resistant to diastase as the original material.

Kerr and Trubell (24) investigated this fraction from defatted corn starch, but could find only about 0.15% of SiO_2 with procedures outlined by Ling and Nanji. Further purification of the product by solution in alkali and reprecipitation by neutralizing with an acid gave products yielding less than 0.04% of SiO_2 on analysis. Silica is evidently an impurity in starch which collects with the less soluble fractions.

It is extremely difficult to free certain starches from nitrogen; *e.g.*, corn and wheat starches. This has led to the assumption that small amounts of protein or its degradation products are in chemical combination with some of the molecules. Samec believed that the protein in wheat starch was in combination with the esterified phosphoric acid. This would account for the lower acidity of wheat amylopectin as compared to potato amylopectin which is protein-free. Samec claimed to have removed the protein from wheat amylopectin with pepsin, whereupon the product behaved quite similarly in physiochemical properties to potato amylopectin.

At first Samec suggested that the protein formed a salt with the dibasic amylophosphoric acid. Later, however, he (106) showed that this assumption was unjustified. Samec and Blinc (83) review the evidence presented by Posternak to show that the nitrogenous bodies in wheat starch may be extracted by treating the gelatinized starch with alcohol. In view of the evident conclusion that no chemical union exists between protein and starch through primary valences, Samec suggests that a union may exist by coacervation. This phe-

nomenon studied in the many researches of Bungenberg de Jong (107) has also been accepted by Koets (108, 109) and by von Przylecki and coworkers (110) to explain the physical, colloidal union between starch and protein.

5. Study of Dextrins. Insight into the chemical properties of starch has often been attempted by degrading starch into its supposed "building units." The latter are intermediate in size between the sugars and the amyloses and have been referred to by the very loosely used term, dextrins. Mention has already been made in the previous sections of the dextrins produced from starch by the hydrolytic action of the common diastases. In addition, dextrinous bodies have been made from starch and studied by means of unusual micro-organisms or enzymes, *e.g.* the Schardinger dextrins, by the use of high temperatures in the absence of water, *e.g.* the hexosans made by Pictet, and by the action of acid, *e.g.* Nageli's dextrins.

Hanes (37) has reviewed the subject of the chemistry of diastatically produced dextrins and the interpretation of results in respect to starch structure. Myrbäck (38) has also explored this field in recent years but his results do not fit into the picture drawn for starch by Hanes whose interpretations are based on the straight chain concept of starch structure. Very recent work by Hopkins and coworkers (111) is also not in accord with Hanes' conclusions. It would appear that some linkages of starch are more readily ruptured by α -amylase than others and that dextrins which are longer than a chain of 6 glucopyranose units form. It would appear that most of the work with diastases requires reinterpretation in the light of our newer knowledge concerning starch composition and structure.

It is supposed that the diastases are composed of two classes of amylase: a dextrinogenic and a saccharogenic factor. The former is often called α -amylase and degrades starch, supposedly, into dextrins of relatively low molecular weight. The latter is often called β -amylase and degrades starch into maltose, possibly some higher sugars, and a dextrin or dextrins of relatively high average molecular weight. Other types exist, such as those which possess α -glucosidase functions and those that are possibly limited in their action to starch "liquefaction," but they are not of concern in the present discussion.

The action of β -amylase is better understood. Apparently only a maltose configuration fits into the enzymic mechanism for hydrolysis (37) and, accordingly, the more linear components of starch are converted to a high percentage of maltose. Conversely, the per cent maltose production by β -amylase has been used as a measure of the proportion of available straight chains in starch fractions. β -Amylase continues to hydrolyze maltose units from the free ends of the side branches of the more complexly constituted fractions until an anomaly in configuration is encountered, as, for example, a point of branching through a 1-6 linkage. The reaction then ceases or is greatly reduced in its rate. The residue at this point is called a limit dextrin (also referred to as residual dextrin). When properly prepared, from either starch or an amylopectin fraction, with pure enzyme, it should contain all the anomalies present in the original substrate.

Most of these anomalies, at any rate, are to be found in the amylopectin fractions of starch.

As stated above, Myrbäck has hydrolyzed these limit dextrins and has reported the recovery of sugars which contain 1-6 α -glucosidic linked glucopyranoses, evidence that the original anomaly in the amylopectin molecule which hindered the enzymic activity is a 1-6 linkage to a side branch. Nakamura (112) has reported finding sugars with 1-3 α -glucosidic linked glucopyranoses (3-[α -D-glucosyl(1,5)]-D-glucose (1,5)), but these results have not as yet been substantiated by other workers.

Caldwell and Hixon (54) have examined these limit dextrins by methylation procedures, but in spite of the fact that all of the anomalies of the parent starch structure should have been concentrated, so to speak, in this fraction, actually less dimethylglucose was obtained after complete methylation and hydrolysis than was obtained from the parent starch.

Most starches give a yield of about 40% of limit dextrins, waxy starches about 46%, and purified amylopectin fractions about 48%. In other words, almost 50% of the total structure of the amylopectins is composed of linear branches. A continuation of this line of study, coupled with related investigations, should eventually develop a structural pattern for a given amylopectin. Knowledge should be gained of the average length of side branches, spacing on the main chain between branches, or possibly information as to whether the structure is an almost endless progression of branches from branches. Meyer (72, 113) has already drawn the conclusions from his work to date that amylopectin patterns, instead of being straight members with several relatively long side branches, are, rather, a maze of branches and side branches.

His conclusions from work with α - and β -amylase, however, and in particular from methylation data are at variance with the work of Caldwell and Hixon referred to above. The latter have taken the percentage of dimethylglucose formed as a measure of branching, whereas Meyer *et al.* estimate the amount of branching by determining the per cent of tetramethylglucose which forms from terminal groups. Just how there may be more than 1 terminal residue per molecule unless branching exists, and how there may be branching without dimethylglucose resulting from methylation and hydrolysis is not clear. Hixon suggests that β -amylase may pass a branched "block." If so, what prevents the enzyme from continuing the conversion to completion? The linear chains are hydrolyzed almost completely by β -amylase. There are several alternate explanations, any one of which, if correct, would not be in accord with the complete concepts of starch structure elaborated by Meyer. It may be possible that branching occurs through a 1-5 glucosidic linkage, in which case no dimethylglucose would form. Hydrolytic scission may have occurred during the methylation procedures used by Meyer and also by earlier workers such as Haworth (36) who obtained results on limit dextrins comparable to those more recently reported by Meyer. The amylopectins may consist of central chains with relatively long side branches to give a shape to the macromolecule consistent with the physical

behavior of amylopectin but requiring few branched points. These long members under various physical stresses and strains that develop might be very vulnerable to hydrolytic scission at some point where strain is greatest and readily produce new terminal groups by such rupture. The initial action of acid on starch, as well as the action of the amylase of *Bacillus macerans*, both of which are to be discussed later, is in harmony with this last view. The very noticeable increase in reducing power of starch under the influence of physical forces, such as in very carefully controlled ball-milling (22), would harmonize with this view. Another concept, discarded in recent years, cannot be overlooked. It may be that amylopectins are composed of linear chains united by bonding which approaches a primary valence bond in the energy required for cleavage and which is more permanent therefore than the hydrogen bond, hydroxyl (to water) to hydroxyl which ordinarily is involved in the association of the otherwise free, linear chains of glucopyranoses. Lastly, the possibility cannot be overlooked that the exact configuration of these dextrans, like the Schardinger dextrans, does not exist in starch, but rather an anomaly in configuration is formed by the enzyme itself after a definite amount of hydrolytic scission has occurred (114). On the basis of such a theory the methylation data of both Hixon and Meyer could be reconciled.

Meyer, himself, has presented interesting evidence to favor this hypothesis. Whereas the phosphorylases of the type which reversibly catalyze the system glycogen (or starch) + phosphate \rightleftharpoons glucose-1-phosphate (such as liver, heart, and yeast phosphorylases) are able to form and degrade the most complexly constituted configurations in starch, supposedly the branched structures, the yeast phosphorylases do not degrade limit dextrin beyond a dextrin stage according to Meyer and Bernfeld (115). Moreover, Meyer finds that yeast phosphorylase acts on 1-6 α -glucosidic linkages.

The theories mentioned as well as other possible explanations should be considered in a review of this highly important phase of starch chemistry.

The action of α -amylase is not well understood and work with this enzyme should be repeated and the dextrans resulting from its activity should be re-inspected, working with the purer components or fractions that are now available. Experiments similar to those reported by Samec and Labernick (116) should be extended. These workers find that α -amylase converts their potato erythrogranulose (related to amylopectin) from spherical particles with a molecular weight of 230,000 to more linear particles of lower molecular weight. Molecular weights were determined osmotically. The form of the particles influences the specific viscosity at a given molecular weight.

The method of approach outlined in preceding paragraphs has been applied by the author to one enzymic degradation which has attracted the attention of many carbohydrate chemists since its discovery by Schardinger nearly 40 yrs. ago. This list includes among others, Pringsheim, Irvine, Freudenberg, Hudson, and their coworkers.

Schardinger (117) reported that a bacterial degradation of starch with *Bacillus macerans* yielded several new products called by him "crystallized dextrins" or "crystalline amyloses." The procedures, as finally developed by Schardinger and later by Pringsheim, consist of inoculating a sterile 5% starch paste with a potato culture of the bacillus and incubating the mash at 45° C. for several days. The fermented liquors are heated, filtered, concentrated, and an excess of an organic solvent added, such as chloroform or trichloroethylene. After refrigeration for several days, the crystalline dextrins separate. These may be fractionated into an α and β series by dissolving in hot water, cooling the solution, and allowing the β -dextrins to crystallize. By evaporating the mother liquors from the β -dextrins to a sirup, the α -dextrins may be induced to crystallize by the addition of methanol.

The many researches of Pringsheim and his coworkers have been summarized by Pringsheim (118). In this review, the possible structure of the dextrins is discussed from a study of their reaction products with acid, from their empirical formulae, and from determinations of the molecular weight of their derivatives. The empirical formulae of each type were given as $(C_6H_{10}O_5)_n$, and the highest number for n in each series was found to be 6. The α -dextrin, called α -hexaamylose, was reported to originate from what was supposedly a preparation of amylose, whereas the β -hexaamylose was shown to result from Pringsheim's preparations of amylopectin. Both, reportedly, were formed from their respective parent sources through a dissociation of secondary valences. However, Pringsheim eventually came to the conclusion that these polyamyloses do not exist as such in starch. He intimated that in the dissolution of secondary valences to form the polyamyloses rearrangements in primary linkages may take place. The reason advanced was that the diastases, which convert starch to maltose, do not produce maltose from the Schardinger dextrins.

Pringsheim collaborated with Irvine (119) in applying the methylation technique to the polyamyloses. Hexa-(trimethylamylose) was prepared from β -hexaamylose and yielded only 2,3,6-trimethylglucose on hydrolysis and gave a melting point depression in camphor corresponding to $(C_6H_{10}O_5)_6$. The conclusion to be inferred is that the configuration is cyclic, since from identical methylation data Irvine (120) had proposed a 6-membered cycle of glucose units as the elementary building unit of starch.

Just how the two units were supposed to vary, if at all, is not clear, for we are left with the ambiguous statement that "if the molecular units of starch be different, they are constructed on the same model."

The interest in the Schardinger dextrins waned with the rise of the straight chain concept of starch and the dextrins were almost forgotten (121-123) until Freudenberg reported his chemical data to support Staudinger's branched chain formula for starch. Thereupon Freudenberg suggested (124) that the nucleus of the starch molecule could very well be a Schardinger dextrin cycle (of about 5 to 7 glucose residues) with side branches emanating from the nucleus. Almost

immediately, however, Freudenberg (125) withdrew his proposal as an improbable one.

Whereas early workers had used the living bacilli to produce the dextrins during fermentation, Freudenberg used a sterile filtrate of the culture media on which the bacilli were grown as a source of the active catalyst. Hudson and Tilden (126) showed the action to be an enzymic one by making preparations of the enzyme from the bacillus and obtaining high yields of dextrins. From the high yields obtained and because the action was shown to be induced by an enzyme, these workers were led to believe that the structures of the dextrins either were present in the parent starch or else configurations preexisted in starch which were so closely related to those of the dextrins that only a very slight change was required for the dextrin structure to be obtained.

Hanes (37) had suggested in his arguments to support the straight chain concept of starch structure that in solution these chains assumed the shape of helical coils. Hence the cyclic dextrins could very well be formed by the enzyme cutting off a loop of a coil and closing it, to form a 6-membered ring of glucopyranoses. Freudenberg elaborated on this possibility (125).

The writer attempted to settle the issue and prove that these cycloamyloses (Schardinger dextrins) are synthesized by the enzyme from the simpler configurations in starch, and that they do not exist preformed in the more complexly constituted portions. Using the enzyme prepared by procedures outlined by Tilden and Hudson, Kerr (114) reported that no Schardinger dextrins are formed by the *Bacillus macerans* enzyme from the limit dextrins left after a β -amylase hydrolysis of starch, and that a preliminary acid hydrolysis of starch materially lessens the yield of Schardinger dextrins which are obtained. Yet β -amylase was found to be without any effect whatsoever on these Schardinger dextrins. The results for highly purified starch fractions are still more conclusive. From the most complexly constituted fraction of corn starch, *i.e.* the amylopectin types, least precipitable by alcohols, such as mixtures of butanol and methanol, which fraction yields limit dextrins to the extent of about 45 to 50% of its weight, a yield of 44% of Schardinger dextrins was obtained. From the most linear fraction of corn starch, crystalline amylose, of which about 94% of the weight is converted to maltose with β -amylase, Kerr (127) obtained yields of cycloamyloses exceeding 70% when precautions were taken to eliminate an undue retrogradation of the substrate from the reaction mixture. Retrograded amylose is apparently inert in the presence of the enzyme.

French and Rundle (128, 129) have recently reexamined the cycloamyloses and have shown that both consist of cyclic arrangements of glucopyranose units, mutually linked by 1-4 α -glucosidic bonds. The α variety consists of 6 members, and the β is composed of 7. They have accordingly been renamed, cyclohexaamylose and cycloheptaamylose, respectively.

It is evident that a very substantial portion of a linear chain or linear side branch can be broken down into these cycles. It is also evident that the side branches of complex amylopectin structures are longer than 6 glucopyranose

units. A most important question is, therefore, how long must a linear chain of glucopyranoses be before the enzyme is able to cut off the first coil to form a cycle? Present indications are (from unpublished data) that this value is in excess of 20. This value would harmonize with the fact that when acid-converted starch solutions containing linear chains (130) as great as 26 glucoses in length are treated with the enzyme (114) only a negligible yield of dextrans results. The figure is also in harmony with the result given that less than an 80% yield of dextrans has been obtained from crystalline amylose. Thus, if no minimal length of chain is required (over 6 glucopyranose units), it would be supposed that the *Bacillus macerans* enzyme should extend its conversion practically to completion, since patently we are not dealing with an equilibrium. When this question is settled, a definite answer may be forthcoming from these studies on the length and relative number of side branches in a branched configuration of known size.

The action of acids on starch is probably more indiscriminate than the action of enzymes, and hence little importance has been attached to dextrans made by the action of acids until recently. If a linear chain of 1-4 α -glucoside-linked glucopyranoses remains extended in the presence of an acid, it is quite likely that the hydrolysis would be perfectly indiscriminate, and at any time during the hydrolysis a mixture of sugars would be present ranging in size from dextrose up to practically the length of the original chain. The theory of random hydrolysis has been discussed by Kuhn (131). If, however, some of these chains are cross-linked by secondary bonds, from hydroxyl to hydroxyl, it might be granted that the rate of hydrolysis would be reduced. Hence, under certain conditions, portions of the chain which are free would appear to be preferentially hydrolyzed as compared to other portions which are associated.

If linkages other than the 1-4 α -glucosidic exist in the chain, it is possible that the action of acid on a free, extended chain might not be indiscriminate, and those bonds which require a higher activation energy would, under certain circumstances, hydrolyze at a slower rate or possibly not at all.

Other factors complicate the use of acid to produce dextrans in studies designed to give reliable information concerning the structure of starch. Most of these effects can be controlled or minimized by exercising proper precautions. For example, the presence of acid catalyzes a secondary reaction, the rejoining of glucopyranose units into gentiobiose (132), and possibly other configurations as well. This secondary reaction can be controlled, among other ways, by noting that the reaction is reversible and, therefore, avoiding a high concentration of glucose at any period of the hydrolysis. By use of a high dilution of starch in weak acid or by stopping the hydrolysis at stages where the glucose content is low are two means that have been employed.

Among the early work in this field, the experiments of Naegeli (133), in which dextrans are made by treating starch with acid, should be recalled. The method employed consists essentially of allowing the intact native granules to stand in contact with acids, in the cold, for prolonged periods. Depending on the length

of treatment, various products develop which have been referred to as Naegeli dextrins. Eventually, however, a residue is left which has the outward physical appearance of the original granules. This latter product is very interesting and has recently been examined by French and Rundle¹⁰ who find that it is composed essentially of short linear chains about 25 glucose units in length, that it is soluble in hot water but retrogrades on standing, and that it can be crystallized under certain conditions by the use of butanol. It is stained red-purple by iodine. The product has been examined by the author who finds that its limit of conversion to maltose by β -amylase is 74.8% of its weight. Hence short unbranched chains are probably not converted to maltose completely (except possibly after extended periods of time).

Water solutions of the product retrograde to a marked extent when their clear solutions are concentrated or frozen. They precipitate as spherocrystals, or birefringent needles, in clusters. The dextrin gives sharp, crystalline x-ray diffraction patterns of the A, B, C, and V types, depending on conditions.

As pointed out by Bates, French, and Rundle (74), the chain is too short to absorb much iodine in the potentiometric titration for linear chains. Obviously then the linear side members of starch fractions which give a titration curve characteristic of a branched chain could be as long as 25 glucopyranose units.

The retention of the "crystalline zones" of the native starch granule throughout its long contact with acid can be explained by assuming that the orientation of these regions is such that it does not permit the entry of the hydrogen ions. The latter are known to induce hydrogen bonding rather than to rupture hydrogen bonds. The amorphous regions of the granule gradually become hydrolyzed, and the soluble products are leached away.

The action of more dilute acid at slightly elevated temperatures has been reported to increase the total percentage, by weight, of components in whole corn starch which behave as linear components in their precipitability by butanol (21). As high as 35% of butanol-precipitable material has been obtained by Peckham and Newton¹¹ from corn starch pretreated with acid as in processes used to make the thin boiling starches of commerce. The effect has been checked and studied in the laboratories of the author.

The increase in the percentage weight of the linear fraction can be explained by assuming that linear side members are cut off of the branched structures by the acid. Another and possibly more likely explanation, however, would be that linear sections are cut out of the branched structures to form more of the linear fraction. This possibility will be dealt with at the close of the section.

Recently, the hydrolysis of starch by hydrochloric acid at higher temperatures has been investigated, with the focus of attention on the dextrins produced. These conversions have been of the type employed in making sirups in industrial practice. A suspension of corn starch at 22° Bé. is made acid to about pH 1.5 to 2.0 with HCl and heated under 30 to 45 lbs. steam pressure for several minutes,

¹⁰ Private communication. See also reference (74).

¹¹ Private communication.

until its copper-reducing value, estimated as dextrose, is about 40% of the solids present. Actually, however, only about 20 to 25% of dextrose has formed, and, under such conditions, reversions and other side reactions are of negligible importance. The sirup is neutralized with sodium carbonate, clarified, and concentrated in a vacuum evaporator.

Kerr and Schink (134) found that, when a sirup made as described above was treated with malt diastase, the reaction proceeded no further than it did when unconverted starch paste was used as substrate. The extent of the reaction was judged by the production of maltose and other fermentable sugars. Actually 65% of the solid material was fermentable when acid conversion was used first, and 73% when no pretreatment was used. It was assumed that the action of acid might be indiscriminate on the 1-4 α linkages, but that these results indicated that other linkages were hydrolyzed at such a reduced rate that practically none which would act as a barrier to the enzymic components which create fermentable sugars was hydrolyzed by the acid during the period of treatment. It was believed, moreover, that these results showed that in the residues remaining after acid and enzyme treatment the proportion of "unusual" linkages to 1-4 α -glucosidic linkages was higher than generally is supposed. The theory that corn starch is composed of different types of components, some essentially linear and some that are branched through unusual linkages and that have linear branches relatively long in proportion to the distances between branches, would harmonize with the results obtained.

The starch which had been treated with malt diastase only was diluted to 12° Bé., made to pH 1.5 with HCl, and heated at 102° C. for varying lengths of time. An acid treatment as long as 2.67 hrs., at 102° C. was used. The true dextrose content of the converted material increased progressively from 8.2% to 49.5%, but no increase whatsoever was noted in total fermentable sugars. The conclusions given above were apparently confirmed. The anomalous links in corn starch are much more resistant to acid than 1-4 α -glucosidic linkages, and hence the former are found in the dextrinous residues after an acid conversion. Even though the indiscriminate action of the acid can cut in between these anomalous links, there are relatively few dextrose units now available or exposed, linked as 1-4 α -glucosides. The increase in dextrose content by acid treatment of the enzyme-converted sirup is due almost entirely to the hydrolysis of maltose and its homologues of low order already present in the sirup after the enzyme treatment, even for as long a hydrolysis period as 2.67 hrs. at 102° C. It is difficult to interpret the above results on the basis of structures proposed to date for "branched" starch molecules.

The more recent work of Levine, Foster, and Hixon (130) may, moreover, require a still different interpretation of the results reported above. These workers have separated the dextrans from an acid-hydrolyzed, corn starch sirup like the one described above. The dextrans were fractionated by alcoholic precipitation into a series of products having regular, decreasing molecular weights. The molecular weights were estimated from an iodine titration and by

Reu values and the results show a fair degree of correlation. Moreover, the dextrinic acids produced by the iodine oxidation reaction were converted to potassium salts and analyzed for potassium. There is an excellent check between the potassium found and calculated. Furthermore, the dextrans were completely methylated and tetramethylglucose estimated by hydrolysis. A very close agreement was found between chain lengths by this end-group analysis, assuming one terminal residue per molecule, and by the other methods. To confirm the surprising result that these chains, varying in average length up to about 25 glucopyranose units, are essentially unbranched, dimethylglucose determinations were made after methylation and hydrolysis. Practically no dimethylglucose was found, 0.3%, by the method of Bell (34).

Several interpretations of these results suggest themselves. Levene, Foster, and Hixon propose that the 1-6 α -glucosidic linkage, supposedly present in parent starch, is broken at the same rate as the 1-4 α -glucosidic link. (Caldwell and Hixon have proposed, as stated above, that β -amylase is not blocked by a 1-6 linkage on the basis of analogous data.) But if these assumptions are correct, what limits the activity of β -amylase, malt, and other diastases, that does not impede the progress of enzymes with α -glucosidase functions (135)?

If we assume that the blocking link is the 1-5 α , to harmonize with the result that no dimethylglucose is found, then we should assume that this linkage is hydrolyzed at a rate at least equivalent to the 1-4 α type, and that the reason the corn sirup dextrans are not completely converted by malt diastase is that after the length of these chains is reduced below a certain level β -amylase is greatly retarded in its speed of attack.

We cannot, of course, discard the theory that some sort of block exists in the original starch on the basis of the last assumption, for obviously amylopectin fractions which behave as though they were many times the molecular weight of amylose fractions should then be hydrolyzed to a higher per cent of maltose than is amylose by β -amylase.

Other alternative interpretations have been given in another section, and they might be considered especially in view of unpublished work of Kerr and Schink which shows that limit dextrans (from the action of β -amylase on corn starch) separated and resuspended in HCl at pH 1.5 and 102° C. are hydrolyzed at a slower rate than the parent corn starch or its long linear chains such as corn amylose. Furthermore, these dextrans give different proportions of the products of hydrolysis at different time intervals and consistently yield sirups of lower fermentability in the early stages of acid hydrolysis than are obtained by a similar treatment of whole starch with acid.

Of the theories proposed to harmonize the several results of the treatment of starch with acid, the following appears as most logical. The side members of the more complexly constituted fractions of starch are possibly as long as 20 to 25 glucopyranose units, on the average; some are shorter, some are very much larger. They may be joined through 1-6 α -glucosidic linkages to other members of the molecule. Owing to the strain imposed by the long side members on

the glucoside unions with the glucopyranose at the point of branching, the action of the acid at first is preferentially to rupture one of these unions, either the 1-4, the 4-1, or the 6-1 linkage, whichever is the weakest of these three weak bonds. This weakness would account for the accelerated action of acid often observed during the early stages of acid hydrolysis, or as expressed by Haworth (46), the reduced activation energy required in the "disaggregation" of starch, which appears to be less than that required by the ordinary (1-4 α -) glucosidic bond. To explain the anomalous behavior of acid on limit dextrins and of diastases on limit dextrins and on acid-made dextrins, we might assume that the 6-1 linkage is the stronger of the two types and is retained in both classes of dextrins. In any event, the resulting dextrin (with acid) may be essentially linear (unbranched), although in the case assumed the dextrin contains a 1-6 linkage in its chain. In such an event, a refinement of exhaustive methylation technique should disclose the presence of a 1-6 linkage which should be revealed by the presence of a small proportion of 2,3,4-trimethylglucose in the hydrolysate of the methylated (acid-made) dextrins.

6. Synthesis. Eventually, considerable may be learned of the fundamental nature of starch by experiments initiated in recent years on the synthesis of various starch-like products, in which enzymes are used. Cori and Cori (136) have reviewed the early experiments on the study of the enzyme or class of enzymes called phosphorylases which catalyze the reversible reaction: starch (or glycogen) + inorganic phosphate \rightleftharpoons glucose-1-phosphate.

The enzyme is apparently widely distributed in nature, although the enzyme obtained from these various sources may not be identical. It was originally shown to be present in extracts of muscle, heart, liver, brain, and yeast. Hanes (137, 138) has reported its presence in a variety of plant tissues, particularly potatoes which are, apparently, an excellent source. The nature of the enzyme and the kinetics of the reaction have been studied by Cori and Cori (139, 140) and by Hanes (141). According to Cori, the reaction, regardless of whether plant or animal phosphorylase is used, is quite similar in many respects. When the enzyme is added to glucose-1-phosphate, polysaccharides develop with characteristics of glycogen or starch. There is an initial lag period before the enzyme develops activity in the liberation of inorganic phosphate. This induction period becomes extended indefinitely as the enzyme is purified, but it may be eliminated by the addition of glycogen or starch, or even maltose, according to Hanes. Cori reports that muscle phosphorylase is not activated by purified maltose. Speculation has arisen as to why the enzyme apparently needs a pattern to start its synthesis, but Cori suggests that a complex between enzyme and carbohydrate is the active catalyst.

The position of the equilibrium of this reversible reaction is influenced by the pH. For example, irrespective of whether equilibrium is approached from one direction or the other, the ratio of inorganic phosphate to glucose-1-phosphate is 5.7 at pH 6, but falls off to 2.7 at pH 7.6. While the addition of glycogen increases the rate of activity, it does not affect the equilibrium point. Cori

explains this by calling attention to the high molecular weight of glycogen which would require the addition of considerably more glycogen than that used in these experiments, 0.2 to 0.5%, to increase substantially the molar concentration. Temperatures between 15° and 30° C. have no effect on the position of equilibrium. With optimal conditions, the reaction is of the first order. The presence of two distinct enzymes, one synthesizing and the other degrading as suggested by Kiessling (142), is apparently an unnecessary hypothesis.

Several apparently less significant effects and coreactions were noted in early work. More recently these observations have proved to be of extreme importance in postulating the mechanism of the carbohydrate synthesis by way of glucose or other sugars or their oxidation products. It was observed that the addition of adenosine-5-phosphate (adenylic acid) is necessary to activate the phosphorylase obtained from mammalian tissues, whereas for enzymes from other sources such as yeast, peas, and potatoes, no activator is required. Glucose-1-phosphate is converted to glucose-6-phosphate by another enzyme which apparently accompanies phosphorylase regardless of source and has been called phosphoglucomutase (143, 137). Although many instances of phosphorylation of glucose are reported in early work, the product is always glucose-6-phosphate.

Kalckar (144) discovered the anaerobic phosphorylation of glucose by extracts of kidney. Colowick, Welch, and Cori (145) also investigated the reaction. Anaerobic phosphorylation is involved in the transformation of fructose, glycerol, pyruvate, and lactate to glucose. When kidney slices are held anaerobically with pyruvate and fumarate, a synthesis of glucose takes place. The synthesis is accelerated by diphosphothiamine according to Barron and Lyman (146). Cori suggests that this synthesis takes place by way of phosphopyruvic acid, since the reverse reaction, glucose \rightarrow pyruvic acid, has been followed. The formation of phosphopyruvic acid has been observed from malate by Kalckar and by Ferdman and Epstein from lactate in the presence of fluoride (147). Glucose-6-phosphate is also formed by the action of an enzyme called hexokinase in the presence of adenosine triphosphate. There is a transfer of phosphate from the latter to the glucose (148).

Originally it was thought that the action of phosphoglucomutase is irreversible (143). It was a significant advance in the study of carbohydrate anabolism and carbohydrate synthesis when Sutherland, Colowick, and Cori (149) observed that the reaction is reversible in the presence of magnesium ions.

The chain of reactions is therefore complete. Glucose or fructose is phosphorylated by adenosine triphosphate, as the source of phosphate, in the presence of hexokinase to glucose-6-phosphate, or the ester originates as such by way of pyruvic acid. The glucose-6-phosphate is converted to glucose-1-phosphate by phosphoglucomutase. The latter is converted to polysaccharides by phosphorylase in the presence of adenylic acid.

Hanes and coworkers (150) have reported that the polysaccharide synthesized with potato phosphorylase is similar to starch. Its x-ray diffraction pattern is almost identical with potato starch. It is colored blue with iodine. It retro-

grades to an insoluble form as does starch. Haworth and coworkers (47) report that the product behaves towards methylating agents in a manner entirely analogous to the behavior of natural potato starch, but that the average length of its unit chain is 3 to 4 times that which is found for potato or cereal starches. Evidence for 1-4 α -glucosidic linkages only were found.

Bear and Cori (151) have reported that the polysaccharide prepared with muscle extract also closely resembles starch in that it is colored blue with iodine, retrogrades, and gives a crystalline, x-ray diffraction pattern resembling that of starch quite closely. However, the latter investigators report that the polysaccharide synthesized with other phosphorylases such as those from liver and yeast gives an amorphous pattern, is very soluble in water, does not retrograde, and in most respects resembles glycogen. It is colored reddish brown by iodine.

From the foregoing it should be evident, in view of the overwhelming evidence showing a chemically heterogeneous composition of natural starch, that neither type of phosphorylase prepared so far has synthesized a product comparable to starch. It would seem obvious that the phosphorylase prepared from potato and muscle extract synthesizes components comparable to the amyloses in that the chain is essentially linear, the products are colored blue by iodine, retrograde or orient into insoluble residues, and give a low ratio of tetramethylglucose after methylation and hydrolysis, whereas the other type of phosphorylase synthesizes amylopectins which are amorphous, give no blue color with iodine, are stable in solution, and will undoubtedly be found to yield a higher ratio of tetramethylglucose. To synthesize a product having the composition of starch (except possibly the waxy varieties, which are pure amylopectin) requires the activity of both types of phosphorylases discussed. Possibly the presence of two distinct enzymes is not required, since muscle phosphorylase has been observed to synthesize both types of polysaccharides, depending on conditions imposed, but at least the two types of enzyme activity are required.

Recent experiments by Cori and Cori (private communication) may indicate that phosphorylase is a single enzyme which synthesizes only unbranched chains. The synthesis of branched structures depends upon the presence of a coenzyme which by itself is inactive. The presence of unbranched chains in starch is evidence that at some period during the growth of the granule the coenzyme is absent. Hixon and coworkers (private communication) find that a phosphorylase is present in the pollen of waxy maize starch which synthesizes only unbranched chains. Beckmann (private communication) and others find that branched structures are more effective as nuclei from which the enzyme may proceed with its synthesis than unbranched chains. If the above mentioned observations are correctly interpreted, then it follows that during periods in the growth of the granule when the coenzyme is lacking, side branches or other parts of the branched structures will continue to grow in an unbranched fashion and it is logical to presume that some of these sections may approach the length of some of the unbranched molecules when synthesis has been completed. These conclusions favor the view advanced in previous sections concerning the existence of various patterns of branching in starch.

7. **Summary.** In summation, it may be concluded that the starches are composed of glucose units united into molecules of various patterns. These structures vary between an essentially linear arrangement of glucopyranoses, mutually joined through 1-4 α -glucosidic linkages, and those which in addition contain some complexity in their structures. The weight of evidence at present leads to the conclusion that the latter is a branch, and that branching is through a 1-6 α -glucosidic linkage. In some cases branching may be quite extensive, so that nearly 50% of the molecule is composed of side chains united to the main portion of the structure. Some branched patterns are complicated, in addition, by the presence of esterified phosphate, the latter again apparently being united with hydroxyls on carbon atom 6.

In any one starch, a distinction may be made between those patterns in which the linear character of the structure predominates (linear polymers) and those in which the molecule behaves as a branched structure. Precipitation of the linear types by the addition of higher alcohols, such as butanol, is a method which leads to a separation of the two types. Some starches undoubtedly contain a small proportion of unbranched molecules. Other extreme examples of starch are noted in which no molecules are present which are unbranched.

The percentage weight of starch which behaves as though it is composed of linear configurations (and the percentage which is predominantly branched) is of the same order of magnitude for the three industrial starches, corn, tapioca, and potato, being about 29, 20, and 25% respectively.¹² The branched fractions of these starches most likely vary one from the other in their pattern. The potato fraction, at least, contains esterified phosphate. The possibility of a structural variation in the three corresponding linear fractions, respectively, requires further study. The waxy starches, which promise to be of industrial importance in the near future, contain only branched types of molecules. Extremes at the other end have been noted, such as lily bulb starch, which contains as high as 35%, by weight, of constituents which are predominantly linear.

Starch molecules of the unbranched type are of relatively low molecular weight and probably contain about 100 to 500 glucopyranose units. Complexly constituted molecules may contain several thousand.

Further research is required to settle the issue as to whether any one starch may be composed of more than two molecular species; that is, whether variable degrees of branching or ramification are present or whether the differences noted in the subfractions of each species are of molecular magnitude only.

The chemistry of the starches in respect to the reactivity of their functional groups will be dealt with more fully in the chapters comprising the section "Reactions."

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¹² See Chapter XVII for a method of estimation.

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SECTION IV. REACTIONS

From a discussion in previous chapters of the composition of starch and of the general configuration of its component molecules, it is apparent that starch may enter into several types of reactions. Being composed of many glucose units united by glucosidic linkages, the molecules may be hydrolyzed by acids at any or all of the points where a glucoside bond occurs. Conversely, condensation may result wherever the molecule ends in an aldehyde group or wherever there is a free, reactive hydroxyl group. The condensation may be with a hydroxyl group on the same or different carbohydrate chain that contains the aldehyde group, or it may be with the terminal aldehyde and an alcoholic hydroxyl group of a non-carbohydrate substance. Or again, the condensation of aldehyde and hydroxyl may be from a non-carbohydrate carbonyl group to a carbohydrate hydroxyl group. The latter type of reaction is discussed below under "Miscellaneous reactions." Some of the other types are treated in the chapters, "Acid hydrolysis of starch" and "Dextrinization." The hydrolysis of starch, and possibly condensations to some extent, may be induced in the presence of rather specific, organic catalysts which are called enzymes. Two chapters are included which deal with starch-splitting enzymes and their modification of starch.

Since the carbohydrate molecule contains alcoholic hydroxyl groups of both the primary and secondary type and at least potential aldehyde groups, the molecule may be oxidized at one or many of these points; the hydroxyl groups are changed to carbonyl groups and the carbonyl groups to carboxyl groups. The ketone carbonyl groups from the secondary alcoholic hydroxyl groups are oxidized to acids with a splitting of carbon to carbon valences. These reactions are discussed in the chapter, "Oxidation of starch." Aldehyde groups and the aldehydic carbonyl groups which result when glucosidic linkages rupture may be reduced to alcoholic groups. A section, "Hydrogenation of starch," is included.

Alcoholic hydroxyl groups may be derivatized to esters, both with organic and inorganic acids, preferably by the use of acid intermediates such as acid chlorides and anhydrides. The hydroxyl groups of starch may be converted to ethers and to alcoholates with metals or their hydroxides. The chapter, "Derivatives of starch," is devoted to a discussion of these types of reactions.

Starch enters into several other reactions; *e.g.*, when starch is treated with hot sodium hydroxide, it is progressively degraded into bodies of low molecular weight such as lactic acid. Starch is also degraded into bodies of low molecular weight by the extensive action of certain oxidizing agents, by very complicated enzyme systems, or by the effect of heat. The latter type of degradation may involve atmospheric oxidation, dehydration, or a combination of the two.

Some of the large scale manufacturing processes are based on several of the many reactions listed. These types in particular will be discussed together with a few other reactions of either technical importance or academic interest.

Some high polymers, such as starch, do not always react in a straightforward or predictable manner, as do bodies of low molecular weight, because they contain molecules that are so oriented, or cross-bonded, that the resultant forces tend to direct the point of attack of the reagent in respect to the many functional groups or linkages in the molecules. The crystallite structure, the extent to which it is disorganized, and the extent to which reassociations take place may exert a pronounced effect on the mode of chemical reactions involving starch. As an aid to a better understanding of starch reactions, this section is prefixed by a chapter on the hydrogen bond in starch.

CHAPTER IX

THE HYDROGEN BOND IN STARCH AS A BASIS FOR INTERPRETING ITS BEHAVIOR AND REACTIVITY

G. V. CAESAR

1. Theory of the H Bond. "It has been recognized in recent years that under certain conditions an atom of H is attracted by rather strong forces to two atoms, instead of only one, so that it may be considered to be acting as a bond between them. This is called the 'hydrogen bond' . . . It is now recognized that . . . the hydrogen bond is largely ionic in character and is formed only between the most electronegative atoms."

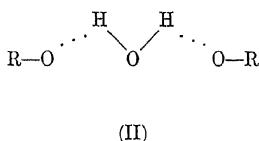
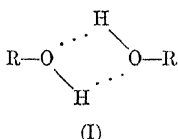
Pauling (1) so characterizes one of the most interesting, and perhaps the most important and far reaching, discoveries of modern physical chemistry. It is a fact now well established by experiments that H can act as a bond between the strongly electronegative atoms, F, O, and N, and, to a lesser extent under suitable conditions, between the weaker electronegative atoms, Cl and C, the link being expressed as $F-H \cdots F$, $O-H \cdots O$, $N-H \cdots N$, $N-H \cdots O$, $O-H \cdots Cl$, $C-H \cdots N$, etc., the dotted link representing the H bond in proton migration. For a more detailed discussion of fundamentals, the reader is referred to the work of Pauling (1). This brief treatise will be confined primarily to the rôle of the H bond in the physical chemistry of starch, with occasional reference to its rôle in the related subjects of cellulose and proteins.

2. Association in Starch. Association of hydroxylated compounds, particularly starch and cellulose, into aggregates or "micelles" has been postulated by various investigators, such as Meyer and Mark (2), and Hirst, Plant, and Wilkinson (3). Others, such as Sidgwick (4), Taylor and Keresztesy (5), Taylor (6), Rodebush and Buswell (7), and Mark (8), have given expression to the thought that the subsequently discovered H bond might play the leading rôle in micellar association. But these intuitive suggestions appear to have been largely overlooked. The result of the consideration of these long chain polymers as molecular entities exclusively, *i.e.* primarily from the point of view of classical

organic chemistry, has perpetuated a poorly defined nomenclature and vitiated some excellent chemical research. It was Taylor's opinion (6) that the study of starch chemistry is incomprehensible without the postulation of micellar association. Direct experimental evidence of hydrogen bonding in starch (and in cellulose), such as has been definitely established for many other hydroxylated compounds by parachor, infra-red absorption measurements, etc., is still lacking, owing to the experimental difficulties involved. But the indirect evidence is abundant and strong. The fact, indeed, that suitable hydroxylated compounds thus far examined, inclusive of water, have invariably shown definite evidence of association through hydrogen, is in itself a very strong basis for a similar association in starch (and cellulose) and warrants careful investigation based on this assumption. Certain investigators of cellulose, *e.g.* Mark (8), Neale and Stringfellow (9), and Lieser (10), and Bath and Ellis (11), who were engaged in protein research, have recognized the probable existence and important rôle of the H bond. In the field of starch chemistry, however, the rôle of the H bond is still substantially unrecognized.

An attempt will be made to follow the behavior of starch in aqueous dispersions, its most characteristic and commercially useful state, and to postulate the association of primary valence chains through the H bond.

In formula (I) the association of 2 molecules of starch in the absence of water



Association of starch (schematic). The H bonds are represented by dots. (I) inactive form; (II) active form.

and (II) the association through water is indicated schematically. Association (I) is of the type now recognized for many carboxylic acids. In a polyhydroxylated compound such as starch, the arrangement would obviously be expected to reduce the reactivity of the hydroxyl groups. Let us consider what happens to starch that is drastically desiccated or thoroughly retrograded. It becomes relatively indispersible (or insoluble) in water. In drastic drying there is evidence that more water is lost than appears to be naturally present, and in retrogradation there is also evidence that intramolecular water is eliminated. In both of these processes we might expect the internal arrangement to approach an association of type (I) in which neighboring OH groups directly associate or are bonded with one another, with consequent reduction in reactivity, or *availability*, of OH groups. In type (II), which may be designated as starch in a normal state, neighboring OH groups (the majority of which may possibly be represented by the primary alcohol groups at carbon atom 6, for reasons discussed elsewhere (12)) are linked through 1 or more molecules of H₂O which pry them apart and render them more reactive.¹ Starch normally contains a relatively large amount of

¹ The O—H ... O distance is 2.76 Å. (1).

available moisture, 10% or more, plus moisture which is emitted only under extreme conditions. On the assumption of association through hydrogen bonding, we should expect the easily expelled water to be loosely bonded to the exterior of the micelles and the balance to be bonded more or less intramolecularly as in (II).²

3. Hydration, Gelatinization, and Gelation in Terms of Changes in H Bonding. Now what may be the mechanism of hydration or gelatinization of normal starch? The hydration or gelatinization of starch is characterized, initially, by swelling of the granular packages, subsequently, by rupture and dispersion of these swollen packages, the degree of rupture and dispersion varying enormously and nearly always exhibiting marked heterogeneity.

Considering the characteristics of the phenomenon of the swelling of starch in aqueous media, it is noted, first of all, that it is occasioned by heating the water or by dissolving certain substances in a cold water suspension of starch. Starch swells slowly and only to a very limited extent in cold water. The probable mechanism which explains the initial hydration of starch is suggested in a recent contribution by Buswell, Gore, and Rodebush (13) on the effect of ions on the coordinated structure of water. The association of water (through H bonds) was studied by means of infra-red absorption. From the results reported, the indications are that the coordinated structure of water is at least in part destroyed by 4 *M* concentrations of salts of the lyotropic series, NaI, NaSCN, NaBr, etc., the effect of the NaI being > NaSCN > NaBr. NaI and NaSCN, among certain other salts, and the caustic alkalies are known to be more or less effective starch-swelling agents in aqueous media. If these agents act essentially to destroy the association of water molecules, that is to reduce the aggregates of water molecules to smaller units approaching in dimensions single molecules of H₂O, it would be expected that the penetrating power of water would be increased, and consequently its ability to become *available* to the tightly packed aggregates of starch molecules would be increased. O—H...O bonds are known to be broken also by heating; hot water is less associated than cold water (14). The conclusion seems probable, therefore, that gelatinization (hydration) of starch is initiated by the more active, dissociated water molecules. It is also quite possible that the same agents which weaken the H bonds between the water molecules also tend to weaken the H bonds through which the starch molecules are oriented in the original granule.

The swelling of the average granule, once started, proceeds with very great rapidity.³ In Figs. 58 and 59 we may follow the record of slow, uniform heating

² The fact that cellulose contains less moisture than starch may be due to its very different molecular form (12).

³ A small proportion of the smaller granules (by weight, not necessarily by number) may be relatively quite resistant to hydration and swell slowly. The writer has recently observed that in nearly all starches there appear to exist large numbers of almost infinitesimal granules which are relatively inert, even in strong reagents. Their nature is still unknown but it is suspected that they may play an important rôle in the behavior of the starch.

and cooling, respectively, of 20% concentrations of a normal corn starch, and of the same material when defatted with dioxane, according to Schoch (15). The assumption of hydrogen bonding clarifies this interesting record. In Fig. 58⁴ the normal starch begins to be hydrated appreciably at about 152° F. and

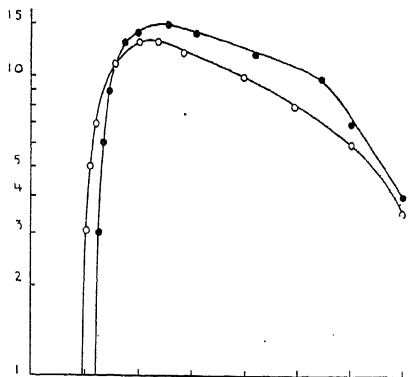


FIG. 58. Gelatinization record of normal and defatted corn starch in Stein Hall consistometer at 20% concentration in water. ● normal corn starch; ○ defatted corn starch. The abscissa, heating temperature in ° F.; the ordinate, consistency in watts.

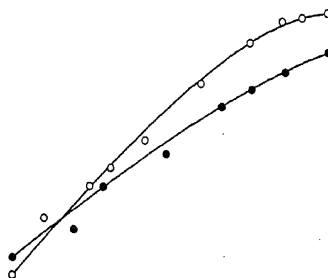


FIG. 59. Thickening record of normal and defatted corn starch in Stein Hall consistometer at 20% concentration in water. ● normal corn starch; ○ defatted corn starch. The abscissa, cooling temperature in ° F.; the ordinate, consistency in watts.

⁴ Figs. 58 and 59 are plotted on semilog paper the better to represent, graphically, changes in all values for paste consistency on a scale which more correctly evaluates the magnitude of such changes.

then swells so rapidly that a consistency value of 9 (9 watts) is reached at about 154.5°. From this point on the percentage rate of increase in consistency begins to decrease rapidly, attaining a peak consistency in the neighborhood of 165°. The swollen granules begin to rupture, under the influence of increasing heat and strong agitation, and spill out clumps of associated complexes which show a wide variation in size, shape, and degree of hydration. This process continues, in addition to the continuation in the swelling process of the more resistant granular packages, through the temperature range of approximately 165–195° F. In the neighborhood of 190–195° a third phase in the gelatinization begins, in which the paste body decreases rapidly as the water temperature approaches the boiling point. Microscopically this phase is characterized by a visual disappearance of organized structure. The latter has become so disintegrated and hydrated that its index of refraction approaches that of water. Actually, the structure is still highly and complexly organized, but it is in a heterogeneous colloidal stage of micellar organization. Further reference will be made to these observations in a discussion of the kinematic viscosity tests. Hydrogen bonds are being continuously broken and reformed throughout the heating period indicated for the consistency curve, the dissociating water molecules force their way into the micellar jungle of bristling, hydroxyl groups which entrap and bind them, as in formula (II), free water is consumed or "bound," and the paste viscosity increased until the peak of the curve is reached. The energy supplied by increasing temperature and the internal shear (from agitation) then begin to destroy the $O-H \cdots O$ links holding the bound water molecules to the starch hydroxyls, and the viscosity rapidly decreases. Prolonged heating also causes more or less depolymerization, an effect which naturally promotes further dissociation, and the loosening of hydrogen-bonded water. Prolonged treatments, by boiling, to decrease paste viscosity in certain commercial applications of starch, however, may cause considerable degeneration of main valence polymers as well.

The defatted starch in Fig. 58 shows several interesting departures from the curve of the untreated starch: gelatinization begins nearly 3° earlier and is well under way before the untreated starch begins to be hydrated; it attains a definitely lower peak, and the decrease in consistency takes place at a more constant rate, showing only a slight indication of the characteristic inflection or shoulder at 190–195°. This behavior is in full accord with what might be expected from assumptions based on the concept of hydrogen bonding: the fatty acids in starch would be attached or spread upon the OH groups by their polar "heads"⁵ in the manner that fatty acids are known to spread upon water; this would tend to impede hydration. It might be anticipated, therefore, that defatted starch would gelatinize earlier and also break down or dissociate more uniformly.

Fig. 59 illustrates the thickening characteristics of these two starches after cooking to 210° F. The defatted starch congeals much faster, and below about

⁵ Schoch (15) shows that the fat is adsorbed on the starch.

110° F. the curve flattens, indicating gel formation. The cooling curve of the untreated starch is more nearly linear, the slope is less, and the gelling tendency less pronounced. This is in accord with the well known paste-softening effect of fats, which interpose long fatty "tails," probably forcing apart the hydrated OH groups, thus promoting a looseness of micellar structure reflected in a softer, more plastic condition at moderate temperatures. In cooling the fat-free starch paste, the slowly reassociating complex of pure R—OH and H—O—H might be expected to become definitely less mobile due to forming a gel.

Thickening, or reassociation, is affected considerably by the rate of cooling, not only for aqueous starch dispersions (pastes) but for modified or degenerated starch dispersions. Rapid cooling increases the fluidity of such pastes; slow cooling promotes the formation of a gel, if such be structurally possible, the water being literally "bound" through a network of H bonds. A rapidly cooled paste is unstable, and tends to thicken progressively on standing, a common and usually unwelcome experience of starch technicians. The paste may be considered as being in a supercooled condition, the most stable hydrogen-bonded structure not having time to become fully developed. The thixotropy of starch pastes, *i.e.* their sensitivity to shearing forces, is well known. The weak, hydrogen-bonded, heterogeneous complex of *partially* disorganized starch and water might well be expected to be unstable to a marked degree. If, however, the starch structure be sufficiently disorganized and homogeneous, a well hydrated paste of such starch would be expected to be relatively stable, both to shearing forces, aging, and small changes in temperature. Its viscous properties would tend to show a similarity to the viscous properties of pure hydroxylated substances. It is known that prolonged mechanical agitation of starch pastes exerts a definite stabilizing effect.

4. Disorganized Starches. The above discussion is further illustrated by Fig. 60 in which the viscous properties in water dispersion of three polyhydroxylated substances, two starches in different states of disorganization and a sample of polyvinyl alcohol, are compared to pure water. From the fundamental equation for viscosity described by Eyring (16),

the plot of the log of viscosity against $1/T$ should be linear, for non-associated liquids. E_{vis} is the "energy of activation for viscous flow." For associated liquids it is not independent of temperature and has a relatively high value, owing to the work required to break O—H...O bonds "before the activated state for flow can be attained . . . As the temperature is raised, there is a decrease in the number of hydrogen bonds that have to be broken before flow can occur, and hence the activation energy decreases . . . For associated liquids . . . viscosities are very much higher than for analogous non-associated substances, and the values decrease rapidly with increasing temperature. The plot of $\log \eta$ against $1/T$ is not linear" (14).

In the study of the viscous properties represented in Fig. 60 the log of the kinematic viscosity was therefore plotted against $1/T$, over the range 283.1–353.1° K. (10–80° C.).⁶ In Fig. 60, Curve 1 represents an aqueous dispersion of a 5% concentration⁷ of a high grade tapioca partially disorganized by thorough

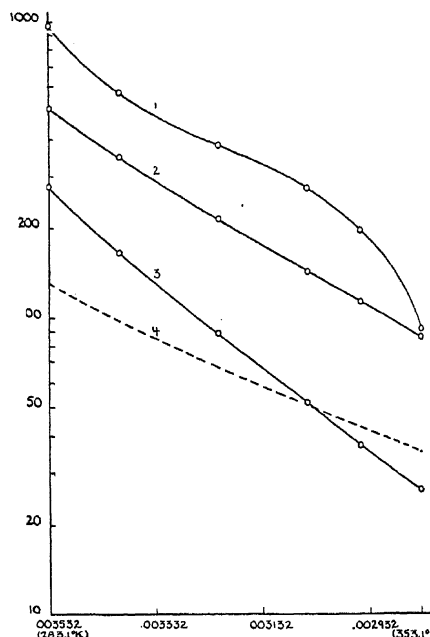


FIG. 60. Logarithmic scale for viscosity in centistokes (ordinate) versus $1/T$ (abscissa) for pastes and water. Curve 1, partially disorganized tapioca paste, 5%; Curve 2, highly disorganized tapioca paste, 10%; Curve 3, polyvinyl alcohol, 10%; Curve 4, water, viscosity scale 1/100.

cooking and rapid agitation in a double action type of commercial mixer. Technically, it would be considered a well dispersed homogeneous fluid paste. Curve 2 represents the same starch at 10% concentration in an exceptional state of disorganization produced by a new process without chemical modification or appreciable degeneration of main valence polymers. Curve 3 represents a sample of polyvinyl alcohol of medium viscosity at 10% concentration. Curve 4 represents, for comparative purposes, the viscosity-temperature relationship of pure water (from data by Bingham), the viscosity scale being 1/100 of the scale for the starches and polyvinyl alcohol. Viscosity values were obtained in accurately calibrated Ostwald viscosimeters of the modified type used by the Socony Vacuum Laboratories, temperatures being controlled to $\pm 0.05^\circ$ F.

⁶ I have found this method of plotting to be very informative and have generally adopted it for viscosity-temperature relationships.

⁷ Kinematic viscosities over a wide temperature range could not be determined at 10%.

It is sufficiently apparent, at first glance, that the structure of the tapioca paste represented by Curve 1 is not homogeneous. Its percentage rate of increase below 80° C. is very great, then decreases, and finally increases again below about 20° C. The structure is a micellar conglomerate, its heterogeneity being strikingly revealed by a plotting of the log of viscosity against $1/T$. Unfortunately, this is the type of starch dispersion too often used as the basis of much laborious research on "amylose" composition.

A very remarkable difference is shown in Curve 2. Obviously, this starch paste approaches complete homogeneity and might be truly termed a solution of "amylose." The plot of the log of viscosity against $1/T$ is remarkably flat, although still by no means linear, and the slope is considerable. Both of these results are strongly confirmative of hydrogen bonding (14).

Curve 3 is what might be expected from a homogeneous long chain hydroxylated polymer of the probable spatial structure of polyvinyl alcohol. It shows more deviation from linearity than Curve 2, and the slope is definitely steeper. Tentatively this might be interpreted to signify the polyvinyl alcohol is more completely and strongly hydrogen-bonded than the disorganized starch (Curve 2).

The curve for water is curiously similar to Curves 2 and 3. Its slope is the least, as might be expected.

5. Location of H Bond in Starch and Influence of Position on Behavior.

The presence of hydrogen bonds in starch is yet to be conclusively proved. By present experimental technique direct proof may well be impracticable. Indirect evidence is accumulating, however. In this connection the recent work of Mullen and Pacsu (17) should be noted. The heat of gelatinization of a number of starches has been studied in water and water-pyridine mixtures. The results indicate that these heats of gelatinization are of the order of magnitude to correspond to the energy required for one to two hydrogen bonds per glucose unit in starch. The theory of H bonds in starch is in sufficient accord with observed phenomena that even now little doubt should remain that starch, like other hydroxylated compounds, is more or less associated through $\text{O}-\text{H}\cdots\text{O}$ linkages. Probably, starch molecules are always more or less associated. This probability should be taken into account in all chemical and physical studies of starch in its many complex manifestations. Unbranched chains, branched chains at the 1-6 linkage, tightly packed or compressed coils or helices (12), extended or strained helices, and the various combinations and proportions of such variables all would affect $\text{O}-\text{H}\cdots\text{O}$ linkages and the physical and chemical properties resulting from such manifold and complex association.

The precise location of the hydrogen bonding in starch molecules is still highly speculative. It seems reasonable to suppose that this location, primary hydroxyls as at carbon atom 6, or secondary hydroxyls as at carbon atoms 2 and 3, depends mostly upon spatial configuration involving the direction of the hydrogen atom, and the oxygen-oxygen distances dependent upon the configuration. This view is repeatedly stressed by Pauling (1) in the discussion of weak and strong H bonds, and it seems particularly plausible when molecules

are constructed from models made to scale (12). It is probable, as has already been suggested, that the primary alcohol groups at carbon atom 6 form the strongest $\text{O}-\text{H}\cdots\text{O}$ bonds,⁸ primarily for the reason that the spatial configuration of the unbranched chain should make them the strongest, an effect particularly potent in promoting retrogradation of unbranched chains. In branched chains, the "wedge" of glucopyranose inserted at the 1-6 position should, on spatial considerations alone, tend to inhibit retrogradation. The fact that unbranched chains show strong retrograding tendencies and branched chains do not is evidence for the configuration hypothesis in respect to the location of hydrogen bonding and, incidentally, for the accuracy of the "picture" projected by recent models constructed. It should be reemphasized that the mechanism of starch association is probably through water molecules (except when the starch is retrograded or drastically dried). In the hydration or the peptization of starch, the thickness of the bound water layers and the uniformity of hydration may vary greatly, depending upon the state of aggregation of the unbranched and branched chains. The secondary OH groups should generally be more occluded than the protruding primary groups, especially in unbranched or so called "linear" chains. In branched chain aggregates, the secondary hydroxyl groups (for spatial reasons) should be more available to attract and build up more relatively stable starch-water complexes. Apparently they are more available and the starch-water complexes more stable (18).

The theory of hydrogen bonding supplies a means for clarifying many incongruities which appear to exist when the chemical structure of the starches is compared with the record of its varied behavior. It is hoped that this incomplete and necessarily speculative treatise may stimulate more use of this recent product of modern physical chemistry to rationalize the results of future research on the chemical and physical properties of the starches.

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⁸ This view is supported by the greater associative tendencies of primary alcohol groups when compared to secondary groups (1) although, as suggested in the text, the association is strongest for the hydroxyl groups of water molecules (Editor).

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CHAPTER X

DERIVATIVES OF STARCH

By ED. F. DEGERING

1. Introduction.

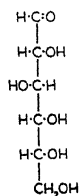
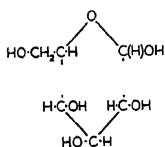
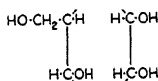
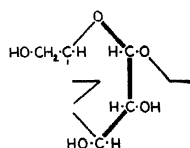
A. Definitions—The expression derivatives of starch, as used in this chapter, refers to compounds or mixtures which retain the intact starch molecule to an appreciable extent. The more important of these, according to studies to date, are the acyl, nitro, and alkyl derivatives. These are also referred to in the literature as starch alkanooates, as starch nitrates, and as starch ethers. The latter terms imply a chemical entity, whereas the former terms allow of greater freedom in defining the product. The author, accordingly, prefers to use the terms acyl, nitro, and alkyl derivatives of starch.

Whereas the definition in the preceding paragraph implies that the starch molecule *tends* to remain intact in the preparation of these various derivatives, it must be conceded that this is only qualitatively true. With techniques now employed to effect acylation, nitration, alkylation, and the synthesis of other derivatives of starch, there is little doubt but that some degradation of the starch molecule occurs.

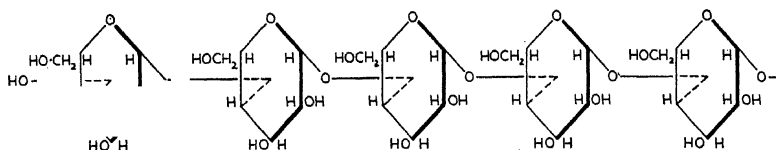
Because of its cheapness and commercial availability, starch has been employed in the past for uses that require a relatively small change in the starch molecule. More recently, however, starch has been examined for possible transformation into compounds in which a major change in its properties may be effected.

B. Structure of Starch—Any consideration of the derivatives of starch requires some discussion of the structure of the starch molecule. While this is dealt with in some detail in Chapter VIII, a very brief consideration of the topic here seems appropriate.

It is now known with a reasonable degree of certainty that (a) the starch molecule is built up of *D*(+)-glucose units (I), (b) these units exist in the pyranose ring (II), (c) the pyranose ring is in the α form (III), and (d) the α forms of these glucopyranose rings are bonded together through oxygen atoms in the 1,4 positions (IV) (34).

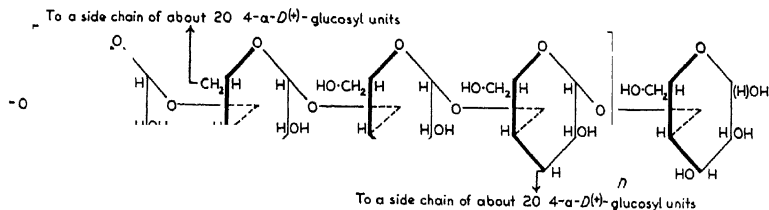
I. *D*(+)-GlucoseII. *D*(+)-GlucopyranoseIII. α -*D*(+)-GlucopyranoseIV. α -*D*(+)-Glucopyranose residue

When the units indicated in (IV) are bonded together, a thread-like molecule of the type shown in (V) is obtained.



V. Thread-like molecule obtained from union of α -*D*(+)-glucopyranose residues through oxygen atoms in the 1,4 positions

Fractionation studies, however, have indicated that starch may be composed of two or more distinct varieties of molecules (103, 107, 136). One of these is generally conceded to be of the continuous chain variety shown in (V), whereas the other is thought to be of the branched chain variety shown in (VI).

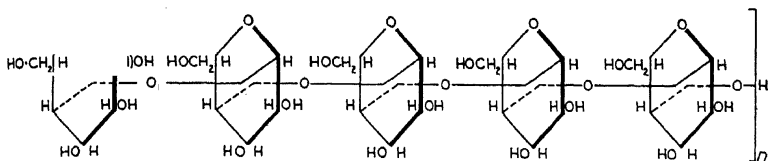


VI. Branched chain molecule obtained from union of α -*D*(+)-glucopyranose residues through oxygen atoms in the 1,4 positions and periodic branching at positions 3 and 6

In view of available data on structure, a mixture of these two general types of molecules present the best current concept of the molecular structure of starch. Corn starch, for example, contains about 70% of the variety of molecule represented by (VI) and 30% of that represented by (V), whereas corn starch from waxy maize is almost entirely of the type indicated by (VI).

C. General Consideration of Derivatives—With the concept of the starch molecule pictured in (V) and (VI) tentatively agreed upon, it becomes apparent that there are from two to four hydroxyl groups per glucose unit available for

such reactions as acylation, nitration, and alkylation. Barring any appreciable amount of degradation during the process of derivative formation, starch might be expected to give two rather well defined series of acyl, nitro, and alkyl derivatives. The one series resulting from the configuration of (V) should resemble rather closely the corresponding derivatives of cellulose, inasmuch as (V) is not too dissimilar from (VII), which represents the generally accepted structure for cellulose. The other series resulting from the configuration of (VI) should have a somewhat different set of properties. That experimental results indicate two such series will be shown in the subsequent discussion.



VII. Thread-like molecule obtained from union of β -D-(+)-glucopyranose residues through oxygen atoms in the 1,4 positions to give the configuration of the cellulose molecule

2. Acyl Derivatives of Starch.

A. Historical Review (23)—The story of the esters of starch has its beginning in 1865 when Schuetzenberger obtained two acetyl derivatives (138). One of these he found to be soluble in both alcohol and acetic acid but insoluble in water. The saponification of both derivatives yielded a dextrin. In 1869, he heated starch with acetic anhydride at 140° C. and obtained an acetyl derivative which was hydrolyzed to regenerated starch (138). Similar results were obtained at 150° C., but at higher temperatures a dextrin was obtained as the hydrolytic product of the acetylated starch. Both the elementary composition and the rotatory power of these derivatives were determined. In 1870, he reported that acetylation of starch at 140° C. yields the triacetate of starch but that at 150° C. the triacetate of soluble starch is the principal product (138). This was confirmed by hydrolysis of the respective products to a starch-like substance and soluble starch.

The use of sulfuric acid as a catalyst in the acetylation of carbohydrates was first reported by Franchimont in 1879 (51).

The oleic-sulfuric acid derivatives of both starch and dextrin were obtained by Liechti in 1883 (88). These products were found to be soluble in water, to yield metallic salts, and to be hydrolyzed with the regeneration of the carbohydrate and oleic acid.

Michael, in 1883, indicated that the starch granule is unaltered upon saponification after acetylation with acetic anhydride, while the presence of even small amounts of acetic acid in the acetylation mixture gives some degradation (100). The use of acetyl chloride was found to give an acylated dextrin.

In 1893, Cross treated soluble starch with alkali and then with benzoyl chloride and obtained the benzoate which he analyzed for carbon (30). Two

years later Boettinger noted the appearance of an ester when starch was treated with an excess of glyoxylic acid (18).

Skraup suggested in 1898 that low temperature and low concentrations of sulfuric acid yield more complex products in the acetylation of starch than are obtained when high temperatures and high concentrations of sulfuric acid are used (139). Syniewski reported during the same year that he obtained an ester by acetylating starch with acetyl chloride and barium carbonate (148).

Pregl, in 1901, treated soluble potato starch with acetic anhydride (115). The derivative had a molecular weight about 10 times that of the empirical formula, had a specific rotation of 163.6° , and was insoluble in alcohol. Hydrolysis regenerated the starch. Acetylation of the same type of starch with an increased amount of sulfuric acid gave a triacetyl derivative. This product had a molecular weight about 3 times that of the empirical formula, melted at 150°C ., was soluble in alcohol, and was hydrolyzed to yield a dextrin.

In 1904, Kldiashvili reported treating starch with formic acid to give the monoformyl ester (85). Cryoscopic measurements indicated a product which contained 6 glucose units. Comparable derivatives were obtained by treating starch with the mono-, di-, and trichloroacetic acids. In the following year he refluxed rice starch with twice its weight of dichloroacetic acid to give a derivative which, according to determinations of molecular weight, contained about 6 glucose units.

Cross *et al.*, in 1905, reported that they prepared the lower acetyl derivatives by heating starch in acetic acid at 100°C . (32). Their work indicated that the extent of acetylation is proportional to the time of heating and the ratio of acetic acid to starch. They described the lowest member of the series as exhibiting the characteristic physical properties of gelatin, and as drying to form a transparent and elastic membrane.

In 1905, Skraup treated starch with acetic anhydride saturated with hydrogen chloride and obtained a number of chloroacetylated starches (139). The chlorine content indicated a minimum molecular weight of 7440.

Law, in 1908, reported that neither acetylation nor hydrolysis occurs when starch is treated with acetic acid and acetic anhydride in the presence of a large excess of zinc chloride, whereas cellulose under the same conditions yields the triacetate (87). In the following year Traquair published a report on the preparation and properties of the formyl and acetyl derivatives of starch (154). He reported, furthermore, on the manufacture, properties, and uses of a product called "feculose," which he prepared by the acetylation of starch with glacial acetic acid or acetic anhydride at 90°C .

A review entitled *Stärkeazetat* was published by Worden in 1913 (163). In 1916 Boeseken *et al.* published on the velocity of acetylation of wheat starch (17). Of the various catalysts examined, they considered hydrogen iodide to be the best. They observed, furthermore, that the rate of acetylation is increased with increasing amounts of sulfuric acid as the catalyst, but that the increase in rate is not proportional to the increase in the amount of sulfuric acid used.

A second review entitled *Stärkeacetat* was published by Halen in 1921 (62). Karrer and Fioroni, in 1922, calculated the heat of esterification of starch to starch triacetate as 1306.0 kg.-cal. (78). Experimentally they found a value of 1296.5 kg.-cal. The heat of combustion of *starch hexaacetate* is reported to be 4499 cal. per gram.

In 1922, Pringsheim and Lassmann treated glycogen and the soluble starch of Zulkowski (degraded by heating in glycerol) with acetic anhydride in pyridine (119). From a consideration of the properties of the derivatives as well as those of the regenerated starting materials, they concluded that starch and glycogen are not identical.

In 1923, Escales and Levy reported that starch is not appreciably degraded when acetylated with sulfo fatty acids (Twitchell reagent) below 80° C. (41). They observed that the acetyl derivative of starch is very hygroscopic and that it is less viscous than the corresponding derivative of cellulose.

Gault, in 1923, reported the preparation of the lauryl ester by treatment of starch with the acid chloride in the presence of pyridine (57). He obtained a water-soluble derivative. Karrer (1923) reported on the treatment of glycerol-degraded starch (soluble starch of Zulkowski) with an acid chloride and quinoline for 4 hrs. (80). He prepared and characterized what he called the starch hexapalmitate and the starch hexastearate.

According to a patent issued in 1923, the higher esters such as the palmitate, stearate, and undecylate may be prepared by treatment of the unmodified starch with the appropriate acyl chloride in the presence of a tertiary base and a diluent under reflux (42). In the same year Berthon described the preparation of the higher esters by pretreatment of the starch with a mixture of benzene and pyridine, followed by heating with the appropriate acyl chloride in the presence of a tertiary base (14). In 1924, a procedure was patented for the production of the linoleic acid and similar esters of starch by treatment of the unmodified starch with the appropriate acyl chloride in the presence of a tertiary base (43).

In 1924, Chowdhury treated starch with chloroacetic acid in the presence of sodium hydroxide and obtained a derivative which contained 2 hydroxy acid residues per glucose unit (27). This product was then methylated with methyl sulfate to give a mixed ether-ester. In the same year Gault prepared and described the diesters of starch (58).

A patent issued in 1926 covers the use of certain esters of starch in coating compositions (96). "Livering" and gelling are prevented by the use of a small amount of an acid such as acetic, citric, formic, malic, oxalic, phosphoric, or tartaric.

In 1927, Bergmann and Knehe degraded potato amylose by heating it in glycerol and then acetylated it by treatment with acetic anhydride in pyridine (8). According to patents issued during the year and subsequently, the higher esters of starch may be prepared by first making a starch paste, then converting to the alkaline starch, and finally treating with the appropriate acyl chloride (45).

Peiser, in 1927, reported the treatment of dry starch with acetic anhydride in the cold to give an acetyl derivative, which was then treated with phosphorus pentachloride in toluene at 105° C. (112). The resulting product was a chlorinated acetyl derivative of starch.

Acetyl derivatives may be prepared by treating alkali-starch with acetic acid vapors, or by pretreatment of the starch with ammonia gas, formaldehyde, pyridine, or steam and subsequent treatment with acetic acid vapors, according to patents issued in 1927 (44).

In 1928, Friese *et al.* published the results of studies in which they degraded potato starch by heating in glycerol and then acetylating the degraded product with acetic anhydride in pyridine (55). In the same year they reported the preparation of the triacetates of both amylose and amylopectin of wheat starch. The derivative of the former was much more soluble in organic solvents than was that of the latter. Other workers have not confirmed these results (20, 150).

Haworth, Hirst, and Webb (65) reported the acetylation of starch with chlorine and sulfur dioxide as the catalyst (Barnett's catalyst (4)). The nature of the final product was not affected appreciably by the temperature of the reaction. Using zinc chloride as a catalyst, Tsuzuki (1928) obtained results which he believed indicated the absence of depolymerization during the acetylation process (156). With the zinc chloride catalyst, he also acetylated starch with acetic anhydride in non-aqueous solvents such as glycerol. He found furthermore, that alkaline thiocyanates act catalytically in the acetylation of starch.

Hess and Smith and also Brigl and Schinle (116), in 1929, reported on the precipitation of rice starch paste with alcohol and the subsequent acetylation of the precipitated product (68). The resulting derivative was found to be soluble in organic solvents. Brigl and Schinle observed, moreover, that the product obtained by treating starch with acetic anhydride in pyridine in the absence of sulfuric acid is different from that obtained by similar treatment in the presence of acid (20). Karrer and von Krauss (1929) precipitated the starch paste with alcohol and acetylated the precipitate to obtain an ester which was soluble in chloroform (79). Patents for the use of sulfur dioxide as an acetylation catalyst were issued during 1929 and 1930 (44). Stein obtained a patent in 1931 for pretreating starch with a liquid swelling agent below 100° C. and then effecting acylation with the anhydride of the desired acid in the presence of a catalyst such as perchloric acid or thionyl chloride (145).

Clark and Gillespie, in 1932, reported the preparation of acetyl derivative of starch by use of glacial acetic acid at the reflux temperature of the solution with or without sodium acetate as a catalyst (28). They observed that a slower reaction rate resulted from the use of sodium acetate. Hughes *et al.* in 1932 reported securing acetyl derivatives readily by the pretreatment of starch with boiling water and subsequent drying with ethanol, followed by addition of acetic anhydride and a trace of sulfuric acid (73). For the production of acetyl derivatives of starch with low viscosities, Snelling and Boyd in a patent obtained in

1932 claim degradation by use of a concentrated solution of ammonium nitrate with a boiling point greater than 110° C. (140).

Maltose octaacetate was obtained by Sutra (1932) when he treated starch with acetic anhydride at 70° C. (147). In 1933, Reich and Damansky reported the treatment of starch with cinnamyl chloride in the presence of pyridine (124). Both the di- and triesters were obtained in good yields. Sutra suggested (1933) that both phosphoric and sulfuric acids cause degradation of the starch molecule (147).

Lorand obtained a patent in 1934 covering the use of sulfonyl chloride or magnesium perchlorate as a catalyst in the production of esters of starch (93). In the same year Damansky indicated that the use of sulfuric acid as a catalyst in acetylation reactions causes degradation of the starch molecule into amylose and biose (33).

In 1936, Genin reported pretreatment of starch by swelling and subsequent acylation by reaction with the anhydride of the desired acid (59). He prepared both stearic and various acetic-stearic esters of starch. The film-forming properties of the mixed esters improved with an increase in the amount of the acetate substituent.

Reich and Damansky, in 1937, claimed that native starch yields the diester and that further acylation results in degradation (123). This claim has not been confirmed by other workers. Staudinger and Husemann (143), in the same year, and also Higginbotham and Richardson, in 1938 (69), reported that, on acetylation of untreated starch, derivatives which are soluble in both chloroform and tetrachloroethane are obtained. The latter workers concluded, moreover, as the result of their studies, that very little degradation takes place through pretreatment with pyridine but that extensive modification occurs when the Barnett catalyst is used in acylation reactions of starch.

Meyer reported in 1942 that corn amylose is readily acetylated to give the triacetate when treated with acetic anhydride in pyridine (97). He found, moreover, that the triacetate from an amylose of medium molecular weight gave a derivative with a molecular weight of 78,000 when osmotically determined in tetrachloroethane. His calculations give a limiting value of 1.7 at infinite dilution for the viscosity of a triacetate of cellulose with a molecular weight of 78,000, and 1.05 for the corresponding value of a comparable starch ester.

B. Preparation of Acyl Derivatives of Starch. (a) *With Acetic Acid, Acetic Anhydride, and Sulfuric Acid*—Acetyl derivatives of starch may be prepared by pretreating 25 g. of corn starch with 25 ml. of acetic anhydride for 30 min., after which a mixture of 100 ml. of acetic acid is added (24). The sulfuric acid, diluted with 45 ml. of glacial acetic acid, is then added. The reaction mixture is allowed to stand at room temperature until the esterification is complete. The ester is then precipitated in cold water, and the precipitate filtered, washed with alcohol, and dried. Some results are presented in Fig. 61.

The effect of time upon the acetyl value of the product is shown in Fig. 62 which indicates an induction period (24). At an acetyl value of about 35 to

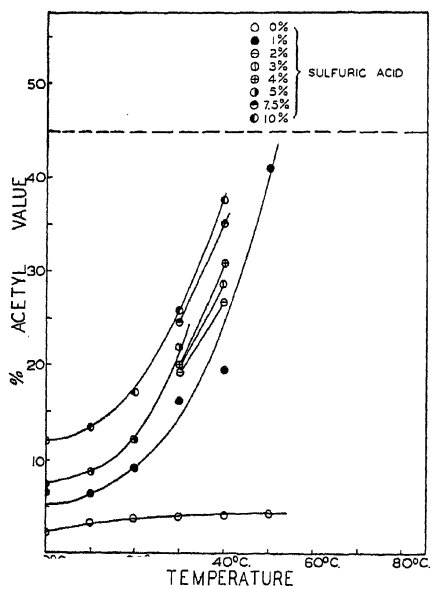
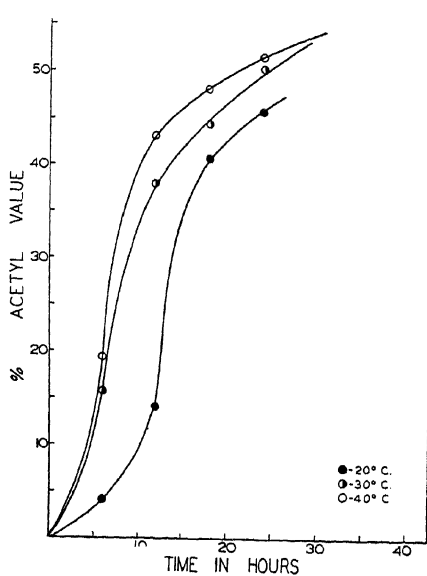


Fig. 61. Acetylation of starch. Effect of H_2SO_4 concentration and temperature on acetyl value.



40% the rate of acetylation begins to decrease. From the graph it is seen that the presence of glacial acetic acid increases the rate of esterification over that reported by Boeseken.

The effect of various catalysts upon acetylation has been studied with regard to yield, reaction time, acetyl value (per cent acetyl, by weight), and product (24). The use of catalysts in the esterification of starch is known to cause degradation (20, 28, 54, 55, 62). In one procedure a small amount of catalyst at the reflux temperature of the acetylation mixture is used. Some results are summarized in Fig. 63. The products obtained after a 24 hr. reaction period, with sulfuric acid and *p*-toluenesulfonic acid, had an acetyl value greater than that of maltose octaacetate. Sodium acetate apparently causes an increase in the rate of acetylation over that obtained without catalysts (*cf.* Fig. 62). This is contradictory to the observations of Clark and Gillespie (28).

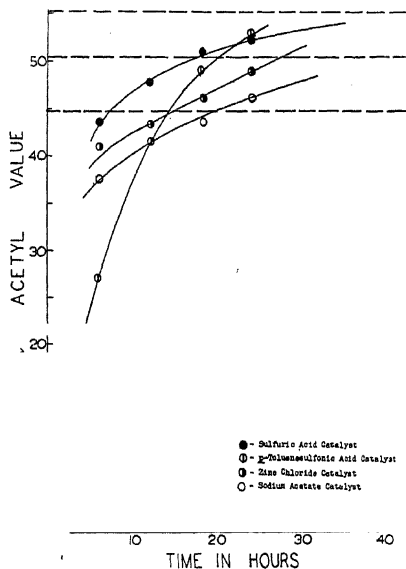


Fig. 63. Acetylation of starch. Change in acetyl value with reaction time when various catalysts are used.

The results shown in Figs. 61, 62, and 63 indicate that (1) the rate of acetylation is increased by the use of acetic acid in the acetylation mixture at both high and low temperatures, (2) the rate of acetylation at low temperatures is proportional to the temperature and the concentration of sulfuric acid, (3) the rate of acetylation increases rapidly after the initial induction period until an acetyl value of about 40% is attained, and (4) sulfuric acid is the most effective catalyst of the four studied.

The method used in measuring the acetyl value of the esters consists in titrating 1.0000 g. of the starch acetate in 50 ml. of carbon dioxide-free water with 0.1 *N* sodium hydroxide until alkaline to phenolphthalein (24). This is a measure of the free acid value. Twenty-five ml. of 0.5 *N* sodium hydroxide are then added, the ester dissolved, and the solution boiled for 5 min. The excess base is neutralized with 0.5 *N* sulfuric acid and the acetyl value of the ester calculated.

(b) *By Gelatinization with Pyridine and Acylation by Removal of Water As a Pyridine-Water Azeotrope*—A suitable procedure is to boil the native starch in water and then add pyridine and continue the boiling with distillation so as to eliminate the water as pyridine-water azeotrope boiling at 92–93°, thereby producing a solution or paste of starch in pyridine (108). The products so obtained range from clear solutions, when the pyridine contains a small amount of water (about 4%), to thick jellies, when the water is absent.

The starch gelatinized in tertiary bases is highly reactive toward esterification reagents such as the anhydrides and acyl halides of aliphatic and aromatic acids and is found to be trifunctional, giving rise to triesters in practically quantitative yield.

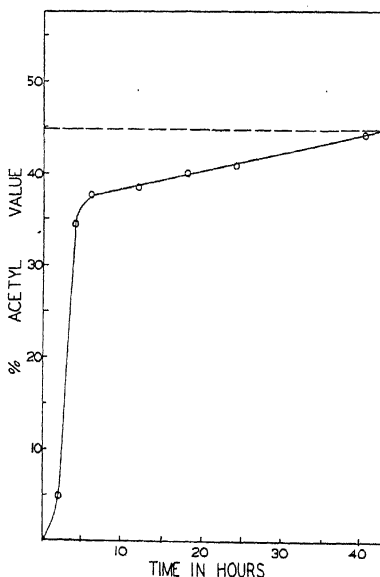


Fig. 64. Acetylation of starch at reflux temperature with no catalyst

After gelatinization, the starch is treated with acetic anhydride and the water of reaction removed by the use of the pyridine-water azeotrope (104). The esters are then isolated by the precipitation procedure. Acetates, propionates, and butyrates have been prepared by this procedure.

To determine acyl content, 0.5 to 1.0 g. samples are placed in a glass-stoppered flask, covered with 10 ml. of methanol, and allowed to stand for an hour (104). An equal volume of water is then added and the mixture made alkaline with 20 ml. of 1 *N* sodium hydroxide. This solution is allowed to stand for 12 hrs., with occasional agitation, and titrated back with 0.5 *N* hydrochloric acid.

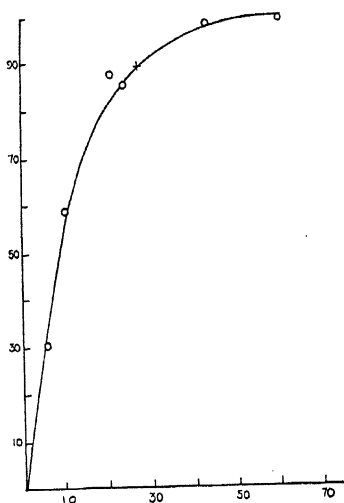


FIG. 65

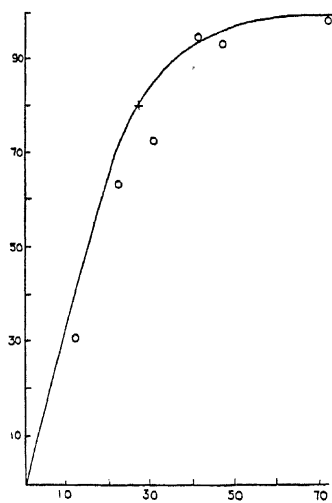


FIG. 66

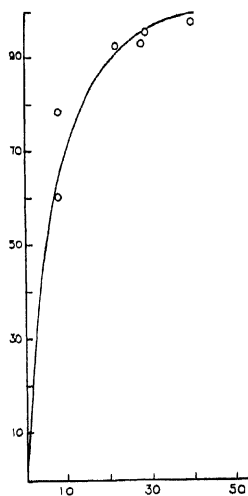


FIG. 67

Figs. 65 to 67. Variation of esterification with time. The abscissa represents time in hours; the ordinate, per cent esterification. Fig. 65, Series I; Fig. 66, Series II; Fig. 67, Series III.

(c) *By Use of an Acid-Anhydride Mixture at Reflux Temperature*—Starch acetates are prepared also at elevated temperatures by refluxing 25 g. of dried starch with a mixture of acetic acid and acetic anhydride (24). Forty-five ml. of acetic anhydride are slightly more than sufficient to react with the moisture in the starch and the water of esterification. An excess of acetic acid is used so that it may function both as a reagent of the reaction and as a solvent for the ester. The product is precipitated in water and leached for 24 hrs., after which it is filtered, washed, and dried. Some results appear in Fig. 64. The rate of acetylation is slow until an acetyl value of about 4% is attained, then very rapid, and finally slow but uniform until acylation is complete. The break in the esterification curve is accompanied by a clearing of the viscous solution. The results of changing concentrations and the lower reaction temperatures in these experiments show that a large excess of acetic anhydride is unnecessary. The lower reaction temperatures give starch acetates of lower acetyl values.

Starch dried at 110° C. has been treated with propionic acid and propionic anhydride under varying conditions with respect to temperature, time, and stirring (94). The results are summarized in Figs. 65 to 69.

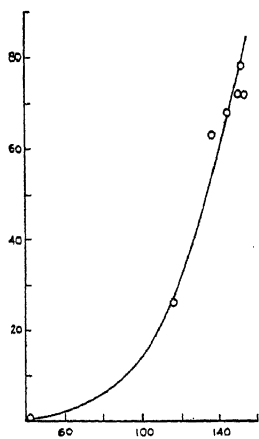


FIG. 68. Variation in esterification with temperature. Abscissa, temperature, ° C.; ordinate, per cent esterification.

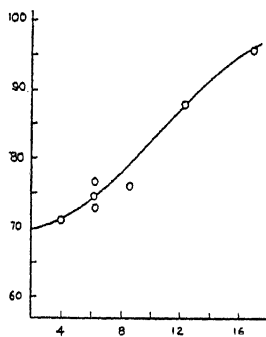


FIG. 69. Variation of esterification with stirring. Abscissa, speed of stirrer, R.P.M./100; ordinate, per cent esterification.

(d) *By Treating Finely Divided Starch with Acylating Agents under Mild Experimental Conditions* (160)—The disintegration of the starch granules is effected by treating swollen starch granules suspended in water to the high shearing action of a Waring blender for $\frac{1}{2}$ hr., at a temperature of about 75° C. This highly dispersed starch is then wholly precipitated by slowly pouring into a large volume of ethanol. The precipitate is then washed with ethanol and dried under a vacuum to a fluffy powder.

The procedure recommended is to suspend 35 g. of fat-free starch in 500 ml. of distilled water and heat with constant stirring in a boiling water bath for 30 min. (160). The hot paste is then disintegrated in the bowl of a Waring blender for 30 min. at 12,000 R.P.M. at a temperature of 75° C. The mixture is cooled to 35° C. and poured into 5 parts of absolute ethanol in a Waring blender. The precipitate is filtered and again dispersed in 300 ml. of absolute ethanol in a Waring blender for three washings. The resulting finely divided precipitate is dried in a vacuum desiccator over anhydrous calcium chloride, and then in a vacuum oven at 70° C. to a moisture content of about 3%.

The starch is weighed (about 50 g.), placed in a round bottomed 3-necked flask, fitted with ground joints, and 2.7 equivalents (4 parts by weight) of dry pyridine and 1.7 equivalents (3.25 parts by weight) of acetic anhydride are weighed in successively (160). A mercury-sealed stirrer is fitted to the center neck of the flask and a reflux condenser protected by a calcium chloride tube is

FIG. 70. Effect of temperature on extent of acetylation of corn starch; acetylation period, 6 hrs. Abscissa, temperature, ° C.; ordinate, per cent acetyl.

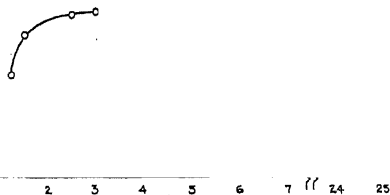


Fig. 71. Effect of time on extent of acetylation of corn starch; temperature, 100° C. Abscissa, time in hours; ordinate, per cent acetyl.

placed in one of the side necks. The third neck is sealed by a glass stopper. The stirrer is started and the flask heated in an oil bath at 100° C. Within 10 to 15 min. the starch dissolves and produces a highly viscous solution. After 3

to 6 hrs. the flask is removed, cooled to about 20° C., and the contents poured into 2 liters of ethanol while being stirred in a Waring blender. The starch acetate which is precipitated in the form of white, curdy flakes is filtered on a hardened filter paper or fritted glass funnel, and again vigorously stirred with a fresh 300 ml. portion of ethanol for three successive washings. The acetate is allowed to stand in 300 ml. of ethanol overnight, filtered, and dried in a vacuum desiccator over calcium chloride. The resulting product is a fine white powder.

The effect of temperature and time on the progress of acetylation of very finely divided starch is shown in Figs. 70 and 71.

(e) *By Use of Ketene on Untreated Starch*—Glucose and some of its derivatives have been acetylated with ketene (74). The acetylation of starch with ketene was studied to determine the type of modification produced by small amounts of ketene. Two general methods of acetylation were used: a liquid phase reaction in ether and in acetone, and a solid gas phase reaction which was carried out in a Pyrex tube (2.5 ft. by 14 mm.). Hydrogen chloride and chlorine were used in several acetylations to increase the rate of reaction of ketene upon the starch in the solid-gas phase acetylations. Table XIV contains the results of these experiments. Tests show that these products have filming properties different from those of starch.

TABLE XIV
Starch Acetate Prepared with Ketene (24)

Experi- ment No.	Solvent	Volume of solvent	Reagent added	Amount added	Temper- ature	Time	Color	Free acid	Acetyl value
		ml.		drops	°C.	hrs.		per cent	per cent
1	Ether	75	H ₂ SO ₄	3	25	0.5	White	0.36	9.43
2	"	90	"	4	25	1	"	0.18	3.72
3	Acetone	70	"	4	25	2	"	0.12	2.18
4	"	80	"	4	56	2	"	0.23	3.52
5	None		None		25	0.5	"	0.12	3.08
6	"		"		25	1.5	Tan	0.39	9.05
7	"		Cl ₂	Saturated	25	2	"	0.32	8.05
8	"		HCl	"	25	2	"	0.07	7.43

C. Properties of Acyl Derivatives of Starch (25)—The acetyl derivatives of starch can be classified into three general groups: (a) water-soluble, insoluble in organic solvents; (b) water-soluble, soluble in organic solvents; (c) water-insoluble, soluble in organic solvents.

The melting point and/or decomposition temperature, specific gravity, optical rotation, viscosity, and solubility are included for six types of standard samples of acetates of starch (*cf.* Tables XV, XVI, XVII).

The melting point and/or decomposition temperature of acetyl derivatives of starch have been reported by several authors (148, 156, 163). In determining melting points, three temperatures were recorded (112): that at which a

TABLE XV
Melting Point, Specific Gravity, and Optical Rotation (25)

Sample No.	Type of ester	Free acid	Acetyl value	Ash	Melting point		Specific gravity, 25° C.	[α] _D ²⁵
					First melting or decomposition	Trans-parent		
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	°C.	°C.		<i>degrees</i>
11	5-7%, low viscosity	0.30	6.60	0.26	240-246		1.64	+225.0*
12	5-7%, high "	0.36	4.90	0.03	235-268		1.59	+220.1*
13	9-11%, low "	0.30	9.30	0.20	238-258		1.55	+188.8*
14	9-11%, high "	0.30	9.80	0.04	250-260		1.57	+180.0*
15	Triacetate, low viscosity	0.20	42.90	0.14	188	205-212	1.24	+166.7†
16	" high "	0.02	42.20	0.02	175	220	1.20	
25	" low "	0.02	41.00		181	200-202		
26	" high "	0.02	41.60		180	195-215	1.15	+172.5†

* Water was used as solvent.

† Chloroform was used as solvent.

pseudoliquid or the first apparent melting occurs, that at which a semitransparent stage begins to appear, and that at which the entire mass becomes transparent. Further heating decomposes the samples. In the case of the water-soluble esters only a decomposition temperature is observed when the samples turn from a white to a brown (*cf.* Table XV).

The specific gravity (Table XV) was determined by means of a 25 ml. pycnometer. Methylcyclohexane was used as the inert liquid (151).

TABLE XVI
Effect of Temperature of Preparation of Solutions of Starch Acetate upon Viscosity

Temperature for preparation of solution	Concentration of solution	Density at 25° C.	Time	Viscosity
°C.	<i>per cent</i>		<i>sec.</i>	<i>millipoise</i>
25	5.12	1.018	113.5	22.58
	5.88	1.021	130.0	26.01
	9.23	1.035	238.5	48.7
	11.73	1.046	360.0	74.5
50	4.64	1.015	112.2	22.23
	9.30	1.035	270.0	55.22
	13.94	1.055	671.0	140.2
	13.17	1.052	500.0	104.2
100	4.65	1.0148	108.8	21.53
	9.54	1.0336	265.0	54.12
	14.07	1.0520	585.0	121.9
	18.72	1.0723	1329	282
	24.69	1.0936	3234	700

Data have been published concerning the specific rotation of acetyl derivatives of starch in various solvents (142-145, 222-223).

Escales and Levy have studied the comparative flow times of acetic acid solutions of acetyl derivatives of starch (41). Staudinger *et al.* have reported the viscosities of some dilute solutions (141-144). Some viscosities of solutions in water, or in acetone determined in an Ostwald viscosimeter, are reported in Tables XVI and XVII.

To determine the effect of age upon the viscosity of solutions of the acetyl derivatives of starch, the pH of the solutions was determined at the time of measuring the viscosity.

To prevent molding, a crystal of mercuric iodide may be added to the water solutions of the derivatives of starch.¹

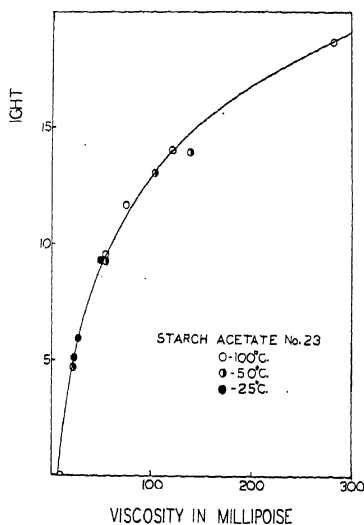


Fig. 72. Effect of solution temperature upon viscosity of starch acetate solutions

The water-soluble esters gave a floc when attempts to dissolve them were made. This was overcome by heating the water to 100° C. before the ester was added and then cooling rapidly as soon as solution was effected. The viscosity of the solutions prepared at 100° C. was the same as those prepared at lower temperatures (*cf.* Fig. 72). All the water-soluble esters were put into solution at 100° C., and no floc appeared even after 5 mos. (*cf.* Table XVI).

¹ Mercuric iodide was found to stabilize the solutions against mold very effectively. Other agents which were tested, but later rejected because of certain physical properties that made them poor agents to add, include formaldehyde, toluene, benzene, acetaldehyde, lead acetate, and xylene.

Viscosities are calculated from the equation

$$n = Adt - \left(\frac{Bd}{t} \right)$$

where n is the viscosity in millipoise, d is the density of the solution, t is the time of flow in seconds, and A and B are constants peculiar to the viscosimeter.

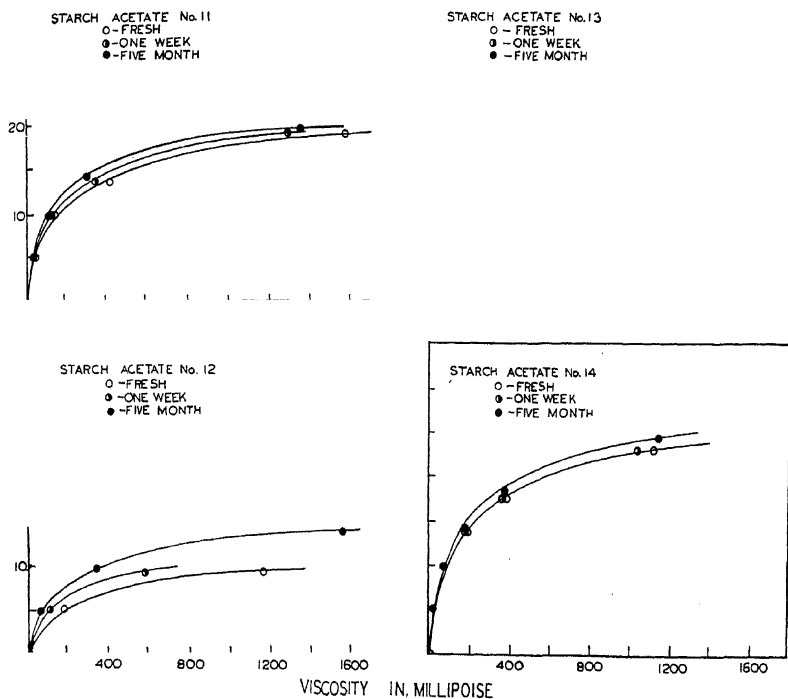


FIG. 73. Viscosity of water-soluble starch acetates

The concentration of the solutions was measured by heating weighed portions of the solutions to constant weight in an oven. The density was measured with a 25 ml. pycnometer. Table XVII contains these results. Figs. 73 and 74 give viscosity data and Figs. 75 and 76 density data. The plots of density against concentration are linear.

As in the case of commercial cellulose acetates and nitrates, the blending of similar acetyl derivatives of starch of known viscosities to prepare a starch acetate of a desired viscosity is possible.

The solubility of the acetyl derivatives of starch in a large number of solvents has been determined by agitating an excess of the ester with 50 ml. of solvent in a sealed bottle in a thermostat at 25° C. for 24 hrs. Those solvents which

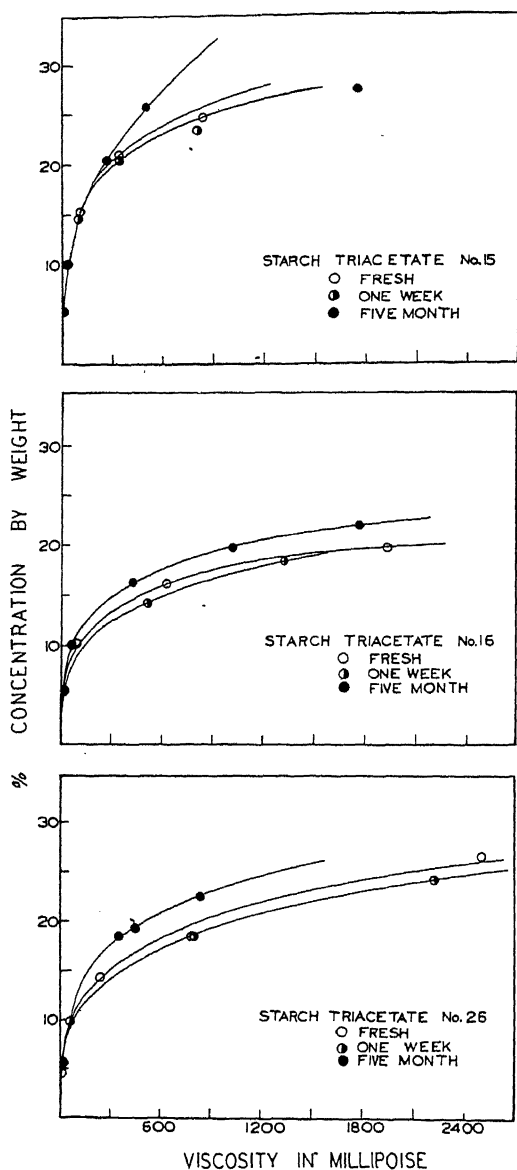


Fig. 74. Viscosity of starch triacetate

· cellulose ni

TABLE XVII
Viscosities and Densities of Starch Acetate Solutions

Starch Acetate No.	Age of solution	Concentration of solution	Density at 25° C.	Time	Viscosity at 25° C.	pH reading
		<i>per cent</i>		<i>sec.</i>	<i>millipoise</i>	
11. 5-7%, low viscosity	Fresh	5.11	1.0173	202.0	40.53	3.95
		9.77	1.0369	714.0	146.6	3.60
		13.45	1.0542	1961.0	410	3.45
		18.80	1.0768	7380.0	1574	3.9
		23.17	1.0870			3.6
	1 wk.	5.11	1.0176	187.0	37.50	3.9
		9.77	1.0360	628.0	128.9	3.8
		13.45	1.0545	1627.0	340	3.7
		18.80	1.0768	6107.0	1299	3.8
	5 mos.	5.13	1.0175	166.2	33.27	3.35
		9.90	1.0288	546.0	111.3	3.25
		14.14	1.0548	1435.8	300	3.20
		19.21	1.0775	6327.0	1350	3.7
		24.01	1.0966			3.2
12. 5-7%, high viscosity	Fresh	5.16	1.0175	896.0	180.6	3.60
		9.18	1.0363	5678.0	1165	3.55
		14.10	1.0569			3.70
	1 wk.	5.16	1.0170	573.0	115	3.8
		9.18	1.0363	2857.0	586	3.5
		14.10	1.0569			3.6
	5 mos.	5.10	1.0180	332.2	66.88	3.15
		9.79	1.0368	1676.4	344	2.90
		13.78	1.0539	7455.0	1556	3.20
13. 9-11%, low viscosity	Fresh	5.56	1.0179	140.2	28.00	3.55
		9.72	1.0357	335.6	68.75	3.5
		13.75	1.0563	828.0	173.2	3.7
		18.28	1.0735	1727.0	367	3.6
		23.33	1.0982	4718.0	1026	3.6
	1 wk.	5.56	1.0189	137.6	27.55	3.8
		9.72	1.0353	320.0	65.55	3.6
		13.75	1.0561	769.0	160.8	3.3
		18.28	1.0735	1587.0	337	3.6
		23.33	1.0981	4396.0	1022	3.5
	5 mos.	5.39	1.0183	133.7	26.68	2.90
		9.77	1.0363	310.6	63.66	2.65
		14.54	1.0710	774.4	162.2	2.80
		18.35	1.0749	1664.0	345	3.0
14. 9-11%, high viscosity	Fresh	5.33	1.0173	142.6	28.50	3.80
		9.74	1.0359	364.0	74.60	3.45
		13.98	1.0542	879	183.5	3.85
		17.68	1.0701	1797.0	381	3.7
		22.99	1.0961	5160	1120	3.6
	1 wk.	5.33	1.0169	139.8	27.95	3.8
		9.74	1.0336	350.0	71.75	3.6
		13.98	1.0541	830	173	3.3
		17.68	1.0700	1710.0	362	3.6
		22.99	1.0965	4794.0	1041	3.5

TABLE XVII—*Concluded*

Starch Acetate No.	Age of solution	Concentration of solution	Density at 25° C.	Time	Viscosity at 25° C.	pH reading	
		<i>per cent</i>		<i>sec.</i>	<i>millipoise</i>		
14. 9-11%, high viscosity	5 mos.	5.25	1.0179	136.8	27.31	2.95	
		9.85	1.0365	353.0	70.33	2.75	
		14.29	1.0545	819.0	180.0	2.80	
		18.50	1.0737	1782.0	379	3.1	
		24.37	1.0975	5290.0	1150	3.1	
Starch Triacetate No.							
15. Low viscosity	Fresh	5.22	0.8042	75.4	11.62	6.1	
		10.16	0.8230	237	38.51	5.7	
		15.17	0.8423		111.1	5.5	
		20.41	0.8665	2016	346	5.4	
		24.73	0.8872	4841	851	5.4	
	1 wk.	5.06	0.8045	75.4	11.62	6.1	
		9.85	0.8248	236.6	38.50	5.6	
		14.49	0.8434	654	109.2	5.4	
		21.03	0.8692	1998	344	5.3	
		23.33	0.8840	4661	816	5.3	
	5 mos.	5.50	0.8058	74.0	11.42	5.9	
		10.37	0.8241	232	37.75	5.4	
		20.34	0.8658	1648	282	5.1	
		25.76	0.8887	2870	505	5.0	
		27.51	0.8963	9900	1770	4.9	
	16. High viscosity	Fresh	5.28	0.8044	140.2	22.13	6.1
			10.13	0.8220	636	103.7	5.7
			16.15	0.8434	3820	638	5.5
			19.70	0.8666	11320	1940	5.4
			5.25	0.8032	136.8	21.57	6.1
1 wk.		9.74	0.8228	645	105.2	3.6	
		14.16	0.8450	3146	526	5.4	
		18.62	0.8646	7781	1333	5.3	
		5.58	0.8065	117.8	18.57	5.9	
		10.52	0.8250	439	71.8	5.4	
5 mos.		16.20	0.8459	2485	439	5.1	
		22.06	0.8732	9910	1715	5.0	
		4.78	0.8027	99.2	15.48	6.1	
		9.69	0.8231	408	66.6	5.7	
		14.18	0.8418	1495	249	5.5	
17. High viscosity		Fresh	18.38	0.8633	4748	812	5.4
			26.87	0.8893	14155	2495	5.4
			4.82	0.8036	99.0	15.47	6.1
			9.83	0.8238	421	68.6	5.6
			14.51	0.8438	1476	246	5.4
	1 wk.	18.60	0.8650	4655	797	5.3	
		24.27	0.8882	12645	2225	5.3	
		5.45	0.8061	105.2	16.52	5.9	
		18.56	0.8571	2118	360	5.4	
		19.32	0.8585	2700	459	5.1	
5 mos.	22.47	0.8742	5745	995	5.1		
	32.81	0.9216			4.9		

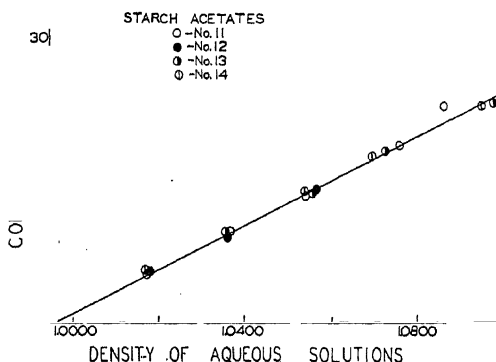


Fig. 75. Density of solutions of water-soluble starch acetates

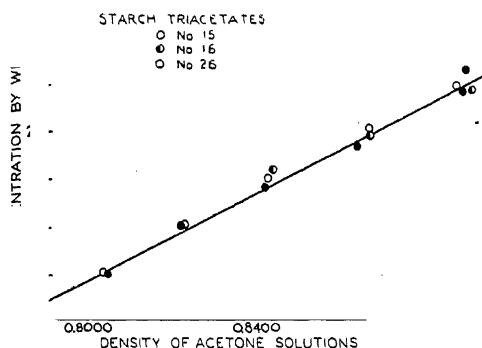


Fig. 76. Density of solutions of starch triacetate in acetone

Some generalizations relative to decomposition temperature, specific gravity, optical rotation, viscosity, and solubility, are as follows:

1. The greater the degree of acetylation, the lower is the decomposition temperature of the ester. The esters of high viscosity have a higher decomposition temperature than the corresponding esters of low viscosity.

2. The specific gravities of the acetyl derivatives of starch are less than that of the original starch.

3. The specific rotation of the esters of starch becomes less as the extent of acylation increases.

4. Mercuric iodide prevents molding of the water solutions of the derivatives of starch.

5. The viscosity of solutions of the acetyl derivatives of starch is less than that of solutions of esters of commercial cellulose. A decrease in the viscosity is

noted after standing for 5 mos., and the magnitude of this change is proportional to the original content of free acid of the solution.

6. The viscosities of the triacetyl derivatives of starch are additive; so that esters of a given viscosity may be prepared.

7. The density of the acetyl derivatives of starch solutions is a linear function of the concentration.

8. The triacetates are not soluble in water, while the water-soluble esters are not soluble in organic solvents.

9. In the case of the triacetates, compounds containing keto groups, ester linkages, halogens as chlorine (except CCl_4), cyclic oxygen compounds, aromatic hydrocarbons and some of their functional derivatives, glycol ethers and their derivatives, and nitro paraffins are good solvents.

10. Ethers, aliphatic alcohols, aliphatic hydrocarbons, and polyhydric alcohols all tend to be poor solvents for the esters of starch.

Starch triacetates have been studied, furthermore, with respect to solubility in plasticizers, compatibility, and plasticizer retention.

Two esters were used, a sample of low viscosity with an acetyl value of 41.0% and a sample of high viscosity with an acetyl value of 41.6%.

The solubility of starch triacetates in plasticizers is included at 25° C., 100° C., and 180° C., 1 g. of the starch triacetate and 10 g. of the plasticizer being used (*cf.* Table XVIII). Corresponding studies of cellulose acetates have been reported by Fordyce and Meyer (50).

Only plasticizers which dissolve starch triacetate at 180° C. are included. The solubility of the triacetyl derivatives of starch in plasticizers correlates very closely with the solubility data for commercial cellulose acetates or nitrates.

Films of acetyl esters of starch, obtained by the method of Reinhart and Kline for films of cellulose esters, were made. The triacetyl derivative of starch, plasticizer, and chloroform were mixed in proportions that gave 7 g. of the triester and plasticizer to 100 g. of chloroform, the ratio of starch triester to plasticizer being 9 : 1. The films were cast on cellophane in Petri dishes (100 × 15 mm.). The results are given in Table XVIII. The films, observed at the end of 1 wk. and 1 mo., are reported as compatible, compatible but cracked, and non-compatible.

Retention of plasticizer by triacetyl starch films upon heating and leaching with water was measured for comparison with the work of Fordyce and Meyer on cellulose acetates (50). All films were heated under a vacuum of about 29 in. at 35° C.

The loss of plasticizer by leaching with water was carried out by placing each film in an individual beaker in a thermostat and leaching at 40° C. for 24 hrs. The water in the beaker was changed at frequent and regular intervals. The films were dried to a constant weight under a vacuum, and the loss by leaching calculated. The films as formed were clear, but, after the leaching, became opaque. Table XIX records retention studies of plasticizers.

TABLE XVIII

Solubility and Compatibility of Starch Triacetate with Plasticizers

S = soluble; M = partially soluble; I = insoluble; C = compatible; P = compatible, but cracked; N = non-compatible; H = high viscosity; L = low viscosity.

Plasticizer	Solubility			Compatibility	
	52° C.	100° C.	180° C.	1 wk.	1 mo.
H					
None				P	C
1. Dimethyl phthalate				C	C
2. Diethyl phthalate				C	C
3. Di- <i>n</i> -propyl phthalate				P	C
4. Diisopropyl "				P	P
5. Dibutyl phthalate				P	N
6. Diamyl "				P	N
7. Dioctyl "				P	N
8. Dimethoxyethyl phthalate				C	P
9. Diethoxyethyl phthalate				P	C
10. Diphenyl phthalate				P	N
11. Dibutoxyethyl phthalate				N	C
12. Benzyl phthalate				P	P
13. Dimethyl sebacate				N	C
14. Diethyl sebacate				N	N
15. Dibutyl "				N	N
16. Ditetrahydro furfuryl sebacate				N	P
17. Monotetrahydro furfuryl monobenzyl sebacate				N	C
18. Dimethoxyethyl adipate				C	P
19. Dicyclohexyl adipate				P	N
20. Butyl cellosolve adipate				N	P
21. Methyl- <i>o</i> -benzoyl benzoate				P	C
22. Ethyl- <i>o</i> -benzoyl benzoate				C	C
23. Benzyl benzoate				N	P
24. Diethyl tartrate				C	P
25. Dibutyl "				C	C
26. Triacetin				P	C
27. Tripropionin				C	P
28. Tributyrin				N	P
29. Ethylene glycol diacetate				P	C
30. Triethylene diacetate				C	P
31. Tetrahydro β -naphthol acetate				N	N
32. Pentaerythritol tetraacetate				P	P
33. Glucose pentaacetate				P	P
34. Sucrose octaacetate				P	P
35. Ethylene dipropionate				C	C
36. Diethylene glycol dipropionate				C	C
37. Ethylene dibutyrate				C	P
38. Ethyl succinate				C	P

TABLE XVIII—Continued

Plasticizer	Solubility						Compatibility			
	25° C.		100° C.		180° C.		1 wk.		1 mo.	
	L	H	L	H	L	H	L	H	L	H
39. Lauryl salicylate	I	I	I	I	I	I				
40. Cyclohexyl laurate	I	I	I	I	M	M	N	N	N	N
41. Chlorinated methyl laurate	I	I	I	I	S	S	P	N	P	N
42. 2-Ethylhexyl laurate	I	I	I	I	I	I				
43. Ethyl crotonate	I	I	I	I	S	S	P	P	P	P
44. Butyl "	I	I	I	I	S	S	P	P	P	P
45. Tributyl citrate	I	I	S	S	S	S	P	C	P	C
46. Acetyl triethyl citrate	I	I	S	S	S	S	P	C	P	C
47. " tributyl "	I	I	I	I	M	M	N	N	N	N
48. Butyl acetyl ricinoleate	I	I	I	I	M	M	N	N	N	N
49. " stearate	I	I	I	I	M	M	N	N	N	N
50. Triethyl phosphate	I	I	S	S	S	S	C	C	C	C
51. Tributyl "	I	I	S	S	S	S	P	C	P	C
52. Tricresyl "	I	I	I	I	S	S	P	N	P	N
53. Triphenyl "	I	I	S	S	S	S	P	C	P	C
54. Plasticizer 2 (Dow)	I	I	I	I	S	S	N	P	N	P
55. " 5 "	I	I	I	I	S	S	P	P	P	P
56. " 6 "	I	I	I	I	S	S	P	P	P	P
57. " 7 "	I	I	I	I	S	S	N	P	N	P
58. " 9 "	I	I	I	I	M	M	P	P	P	P
59. Santicizer 8	I	I	S	S	S	S	P	C	P	C
60. " 9	I	I	I	I	S	S	P	N	P	N
61. " 10	I	I	I	I	S	S	P	N	P	N
62. " B-16	I	I	I	I	S	S	P	P	P	P
63. " E-15	I	I	S	S	S	S	P	P	P	P
64. " M-17	I	I	S	S	S	S	P	C	P	C
65. Sanolite K	I	I	I	I	S	S	P	C	P	C
66. " MS	I	I	I	I	S	S	P	C	P	C
67. " MHP	I	I	I	I	S	S	P	C	P	C
68. Benzophenone	I	I	S	S	S	S	C	C	P	P
69. Acetamide	I	I	S	S	S	S	N	P	N	P
70. Acetanilide	I	I	I	I	S	S	P	C	P	C
71. Hercolyn	I	I	I	I	S	S	N	N	N	N
72. Abalyn	I	I	I	I	S	S	N	N	N	N
73. Sipalin AOM	I	I	I	I	S	S	P	N	P	N
74. Cyclonol	I	I	S	S	S	S	C	C	C	C
75. Triphenyl guanidine	I	I	I	I	S	S	P	P	P	P
76. Camphor	I	I	I	I	S	S	P	C	P	P
77. N,N'-Diethyl	I	I	S	S	S	S	P	C	P	C
78. Paraplex 5-B	I	I	I	I	S	S	N	N	N	N
79. " RG-2	I	I	I	I	S	S	P	N	P	N
80. " G-20	I	I	I	I	S	S	P	N	N	N
81. Flexol-Plasticizer 3-GH	I	I	I	I	S	S	P	C	P	C
82. " 3-GO	I	I	I	I	S	S	N	N	N	N

TABLE XVIII—*Concluded*

Plasticizer	Solubility			Compatibility	
	25° C.	100° C.	180° C.	1 wk.	1 mo.
	H	H	H	H	H
83. Butyl acetal		I	I		
84. Ethyl acetanilide		S	S		P
85. Ethyl- <i>p</i> -toluenesulfonate		S	S		P
86. Diphenyl ether		M	M		N
87. Polychlor naphthalene, 93°		I	I		
88. " " 123°		I	I		
89. " " 135°		I	I		
90. Plasticizer E-30		S	S	N	N
91. " E-40		S	S	N	N
92. " E-50		S	S	N	N
93. " E-60		S	S	N	N
94. Aroclor 1242		S	S	P	P
95. " 1248		S	S	C	P
96. " 1254		S	S	P	P
97. " 1260		S	S	N	N
98. Soybean oil		I	I		
99. Linseed "		I	I		
100. Cottonseed oil		I	I		
101. Castor oil		I	I		
102. Soyapole 75		I	I		
103. Ditetrahydro furfuryl maleate		S	S		
104. Carbowax 1500		S	S		
105. " 4000		M	M		

Some generalizations concerning solubility in plasticizers, compatibility, and retention of plasticizer are as follows:

1. Naturally occurring vegetable oils do not dissolve the triacetyl esters of starch.

2. Esters of low molecular weight are good solvents.

3. Esters of high molecular weight are poor solvents, needing higher temperatures for complete solution.

4. In the cases in which very large viscosities are found to occur in a plasticizer, the rate of solution is impeded.

5. The loss of weight by heating is higher, in general, for triacetyl starch films than for cellulose acetate films.

6. The loss of weight by leaching is also greater for triacetyl starch films than for cellulose acetate films.

7. Triacetyl starch films become opaque and extremely brittle when leached.

3. Alkyl Derivatives of Starch.

A. *Historical Review*—Lilienfeld, in 1912, conceived the idea of producing ethers of starch and secured a patent to protect his invention (89).

In 1920, West published a paper in which he considered the raw materials and the procedure for the production of the ethers of starch (159). He characterized some of the ethers he obtained. In the same year Bayer obtained a patent for the manufacture of the hydroxy ethers by the reaction of the alkene oxides on starch (5). A similar patent was granted to I. G. Farbenindustrie. Dreyfus, in 1920, also obtained a patent for the production of hydroxy ethers of starch by use of such reagents as ethylene chloride, ethylene chlorohydrin, epichlorohydrin, and similar chemicals in the presence of appropriate condensing agents (37).

In 1921, Gomberg treated different starches with benzyl chloride in the presence of sodium hydroxide (60). He observed that the colloidal and paste properties of these derivatives might warrant their use as industrial products. Other patents were secured by Lilienfeld during 1921 for the production of the ethers by treatment of starch with alkyl halides under appropriate conditions (90).

Gault (1923) treated starch with lauryl chloride in the presence of pyridine and toluene at 100° C. for 2 hrs., and obtained an 80% yield of the dilaurate upon precipitation by alcohol (57). He reported that this ether is soluble in benzene and chloroform and that it is non-inflammable. During the same year, Tomecko and Adams reported that they had treated both corn and potato starch with allyl bromide in the presence of aqueous alkali (153). They obtained and characterized both a monoallyl corn starch and a monoallyl potato starch.

Helferich and Koester, in 1924, treated starch with chlorotriphenylmethane in pyridine to give the corresponding ether (67). This ether swells in organic solvents and is readily decomposed by water to regenerate the starch and give hydroxytriphenylmethane.

Schmid and Zentner, in 1928, described a procedure for the preparation of methylated starches by treating starch with diazomethane (134). During the next few years various workers obtained patents for methods of producing the alkyl ethers of starch. Lilienfeld, for example, obtained additional patents in 1929 and 1930 (91).

In 1934, Maksorov and Andrianov reported on the benzyl ethers of different starches and compared the properties of these derivatives (95).

Ziese, in 1934 and 1935, treated starch with ethylene oxide in alkaline solution and obtained the corresponding hydroxy derivatives of starch (167). During the same years, patents were assigned to du Pont for the production of ethers of starch by use of sodium alkyl sulfates as the alkylating agents (39). The patents claim that a variety of products is obtainable by certain variations in the experimental conditions.

Pancirolli (1937) treated alkali starch with *p*-aminobenzyl chloride (109). Such a compound may be utilized in diazotization and coupling reactions.

Much of the work on the methyl derivatives of starch has been by those who were interested in the structure of the molecule (52, 53, 70), but the preparation of the higher derivatives has usually been inspired by a quest for important new commercial products.

B. Preparation of Alkyl Starches—The methyl derivative may be prepared rather satisfactorily by the procedure given by Karrer (77). The ethyl derivative may be obtained by the directions outlined in the patent of Lilienfeld (89). The propyl derivative is mentioned in the literature (166), but no procedure is outlined for its preparation. A method recently developed for obtaining the propyl ether of starch is as follows (34):

Thirty g. of corn starch (10 to 15% moisture), 60 g. of solid sodium hydroxide, 450 g. of a 40% solution of sodium hydroxide, and 277 g. of *n*-propyl chloride are agitated in a stirred, heated autoclave for 24 hrs. at a temperature of 135–140° C. A gray solid is obtained on steam distillation of the reaction product. This solid is readily purified by dissolving in glacial acetic acid, filtering, and precipitating with water, or by pouring the acetic acid solution into violently agitated water. The white product which floats to the surface is filtered, washed with sodium carbonate to remove acetic acid, and then thoroughly washed with distilled water. The yield of the air-dried product is about 30 g.

The butyl ether of starch may be prepared by the following method (34):

15 g. of corn starch, 250 g. of 40% sodium hydroxide, and 160 g. of *n*-butyl chloride are rocked in a heated bomb for 24 hrs. at 165–170° C. The product is steam-distilled to remove butyl chloride, butyl ether, and butyl alcohol. After cooling, a compact, brittle solid is obtained which may be purified by use of glacial acetic acid (*cf.* propyl derivative). The product is a white, fluffy powder which is not plasticized by acetic acid during the purification process. About 18.4 g. of the alkylated product are obtained. Higher temperatures or higher alkali concentrations must be avoided to prevent the formation of butyl ether which is tenaciously retained by the alkylated product.

C. Properties of Alkyl Starches—The methyl derivative of starch is water-soluble and therefore must meet the competition of cheaper starch products. The ethyl derivative of starch can be made both as water-soluble and insoluble derivatives. One alkyl group per glucose residue produces solubility in water, whereas two ethyl groups give a water-insoluble product. The latter product is more soluble in organic solvents.

TABLE XX
Solubility of Alkylated Starches

Solvent	Ethyl derivative	Propyl derivative	Butyl derivative
Acetone.....	Soluble	Soluble	Soluble
Toluene.....	"	"	"
Amyl acetate.....	"	"	"
Butanol.....	"	"	"
Petroleum ether.....	Insoluble	Partially soluble	Partially soluble
Tetrachloroethane.....		Soluble	Soluble
Ethyl alcohol (95%).....	Soluble	"	Partially soluble

The propyl derivative of starch is definitely water-insoluble when two propyl groups per glucose unit have been introduced into the molecule. The butyl derivative of starch is even more like the non-polar type of organic compound. The solubility characteristics are given in Table XX.

These alkylated derivatives of starch are compatible with commonly used plasticizers such as dibutyl phthalate, tributyl phosphate, Ethox, Flexol, tributyl citrate, and Herculyn.

Because of the nature of the compounds, no accurate melting points have been obtained for these derivatives. The observable physical change can be described as a shrinking or softening. The points of softening (uncorrected) for the alkyl starches are as follows: for the ethyl derivative, 140–145° C.; for the propyl derivative, 105–110° C.; and for the butyl derivative, 73–76° C.

TABLE XXI
Specific Gravity of Alkyl Starches

Derivative of starch	Specific gravity	Temperature
Ethyl.....	1.14	27
Propyl.....	1.05	28
Butyl (kerosene used as liquid)	0.88	28

As might be expected, the specific gravity of the alkyl ethers of starch vary with the length of the alkyl group introduced (Table XXI). The products have about the same specific gravity as the corresponding alkyl ethers of cellulose, and hence have a large spreading power in comparison with that of more dense materials such as the acetate or nitrate of starch or cellulose.

TABLE XXII
Relative Viscosity of Alkyl Starches

Derivative of starch	Time	Viscosity
	<i>sec.</i>	<i>centipoises</i>
Ethyl.....	586	2.92
Propyl.....	390	1.97
Butyl.....	210	1.06

The viscosity of these alkylated products varies inversely as the chain length of the alkyl group. The higher alkyl derivatives require more drastic means for their preparation, and this is reflected in the degradation which contributes to the low viscosity. Table XXII contains the viscosity in centipoises of these derivatives as determined in a 5% toluene solution with the Ostwald viscosimeter.

4. Nitric Acid Derivatives of Starch.

A. *Historical Review*—The first attempt to prepare the nitric acid esters of starch appears to have been in 1833, when Braconnot treated starch with concen-

trated nitric acid and obtained a product which he termed xyloidine (19). He observed that textiles impregnated with xyloidine and then dried were found to retain their stiffness and impermeability even after treatment with boiling water. In 1844, Ballot described in detail the preparation of xyloidine and gave some consideration to its constitution (3).

Pelouze, in 1846, examined xyloidine and pyroxylin critically and found that they differ with respect to both their chemical composition and physical properties (113). He considered, furthermore, the probable utilization of such derivatives of starch for military explosives.

In 1847, Kindt reported a microscopic study of the transformations which accompany the reaction of mixed acid (nitric and sulfuric acids) on starch (84). Payen (1847) verified the preparation of xyloidine by earlier workers, and observed that the starch granule consists of an interior and an outer portion (111). The inner portion, he observed, gives an intense blue coloration with iodine, whereas the outer portion does not. In 1849, Reinsch made a detailed report, which is available in the Columbia College of Pharmacy, on starch nitrate (127).

The regeneration of starch from xyloidine was reported by Béchamp in 1853 when he treated this ester with certain ferrous salts (6). In 1860, he described the nitric acid ester of both dextrin and starch (6). In the following year (1861) Ritter described the preparation of the nitrate of starch and discussed its explosive properties (128).

Béchamp, in 1862, obtained a nitric acid ester of starch (6.7% N) which was soluble in aqueous acetic acid but insoluble in the usual organic solvents (6). He obtained also a starch nitrate which was soluble in acetone, alcohol, and ether, and contained about 11% nitrogen. He decomposed all of these by the action of ferrous chloride to regenerate the starch and liberate nitric oxide. The rotatory power of the various nitrates of starch were observed and recorded.

Berthelot reported in 1876 the amount of heat evolved when starch is treated with concentrated nitric acid to give xyloidine (13). In 1885, he reported further studies on xyloidine.

The history of the preparation and properties of the nitric acid esters of starch was reviewed by Muelhaeuser in 1892 (102). At this time he reported the preparation of esters that ranged in nitrogen content from 10.5 to 13.5%. He showed, furthermore, that these derivatives are not nitro compounds but true esters.

Will, in 1898, nitrated starch by two different methods to obtain products which contained from 13.9 to 14.04% nitrogen (161). These derivatives were soluble in both alcohol and ethyl acetate. They were comparatively stable at 50° C. but decomposed with explosive violence at 194° C. He considered these products to be the hexanitrites of starch.

In the following year (1899) Brown and Millar prepared nitrates of starch containing from 7.8 to 11.5% nitrogen (22). Determinations of molecular weight by lowering of the freezing point of acetic acid gave values around 987. When these derivatives were treated with ammonium sulfide, soluble starch was

regenerated. Sapozhnikov, in 1903, reported the preparation of nitrates of starch (13.4% N) by two different methods (132). Molecular weight determinations by the ebulliometric method gave a value of 1845.

The nitric acid ester of starch was decomposed by Berl, in 1908, by treatment with alcoholic sodium hydroxide (12). The reaction mixture was acidified, and a product obtained whose osazone resembled that obtained from oxypyruvic acid. Traquair, in the following year (1909), published the results on the preparation and properties of the nitric acid esters of starch (154).

Four different varieties of starch were nitrated by Berl and Bütler in 1910 (10). They treated the starch with mixed acid and obtained products which contained from 12.86 to 13.85% nitrogen. These derivatives were studied with respect to solubility in alcohol and ether, hygroscopicity, ignition point, and viscosity. They observed that the viscosity of a 5% solution of cellulose nitrate is about 9000 times that of a corresponding solution of starch nitrate.

Cope, in 1917, reported comparative determinations of the nitrogen of starch nitrate by use of the nitrometer and the nitro method (29). In the same year (1917) Sadtler described in some detail the preparation and properties of nitrates of starch which contained from 12 to 13.3% nitrogen (129).

A patent on the production of starch nitrates was secured by Anchors in 1920 (2). In 1922, Kessler and Röhm presented a critical review of the nitration processes and the properties of the various nitric acid esters of starch (83). Another discussion of the nitrates of starch was published by Okada in 1927 (105).

In 1935, Berl and Kunze published a paper entitled *Zur Kenntnis der Stärkenitrate*, in which they considered the nitration of starch, the morphology of starch nitrate, and the viscosity of starch nitrate (11).

B. Preparation of Nitric Acid Esters of Starch—Starch was nitrated by Berl and Kunze under different conditions (11). They found that mixed acid gave a better product than did concentrated nitric acid, and a nitric-phosphoric acid mixture gave better results than did mixed acid. The phosphoric acid, in the latter case, functions much as the sulfuric acid in mixed acid and becomes fixed in the resulting product to the extent of 0.19% or more.

The esters obtained under these conditions contained $13.0 \pm 0.1\%$ nitrogen, whereas the theoretical value is 14.14%. These workers believe that values in the literature of about 14.10% nitrogen for nitrates of starch are erroneous, or else the starch was carefully fractionated and purified before nitration. Maximum nitration was effected in about 30 min.

When completely dry starch is treated with 100% phosphoric acid at 0° C., there is no apparent action. Upon the addition of ice-cold nitric acid to this mixture, nitration sets in almost instantly, and the nitrogen content reaches a maximum in about 10 min. The resulting product is very stable and has a viscosity much higher than that of the derivative obtained from the action of either concentrated nitric acid or mixed acid on starch.

C. Properties of Nitric Acid Esters of Starch—The viscosity of the nitrates of starch is inversely proportional to the extent of the degradation of the molecule.

Nitration with mixed acid gives a maximum viscosity at the end of about 1 hr. At the end of 12 hrs. the viscosity has decreased to about one-half of the maximum. Much less degradation is effected with the nitric-phosphoric acid mixture, as evidenced by the fact that the maximum viscosity of the reaction mixture is attained at the end of about 12 hrs.

The viscosity of the nitrates of starch prepared in the presence of phosphoric acid was about 10 times as great as comparable derivatives prepared in the presence of sulfuric acid. These results check with similar studies made on the nitrates of cellulose.

The viscosity of a solution of the nitrates of starch with identical nitrogen content seems to be dependent upon (a) the composition of the mixed acid used in effecting nitration, (b) the grade of the starch used, (c) the time of nitration, (d) the temperature at which nitration is effected, and (e) other variables.

Among the variables may be mentioned pretreatment. The data indicate that the viscosity of the nitrated product of a pretreated starch is less than that of the comparable nitrate of the untreated starch. Decrease in viscosity definitely indicates a decrease in the molecular weight of the parent starch molecule. Qualitatively, moreover, the viscosity of the nitrates of starch increases with an increase in the grain size of the starch granule.

When starch is boiled under pressure, there is a decrease in viscosity to a minimum, but when this starch is nitrated there is an increase in viscosity. A

TABLE XXIII

Analysis of Two Comparable Nitrates of Starch

Type of nitrate	Nitrogen	Viscosity at 20° C.
	<i>per cent</i>	<i>poises</i>
Amylopectin	11.82-12.05	28,000-31,000
Amylose	13.40-13.45	61-84

TABLE XXIV

Effect of Precooking under Pressure on Viscosity

Temperature	Pressure	Viscosity of nitrate
<i>°C.</i>	<i>atmospheres</i>	<i>poises</i>
90	0.715	48,000
100	1	38,000
110	1.4	37,140
120	2.0	34,760
130	2.7	28,100
150	4.7	1,600
160	6.1	42
170	7.8	14
180	10.0	6

value of 28,000 to 31,000, for example, is to be compared with a value of 48,000, as shown in Tables XXIII and XXIV.

The nitrates of starch are relatively unstable, but the product obtained from a nitric-phosphoric acid mixture on starch is much more stable than that obtained from mixed acid. Since the phosphoric acid esters of starch are much more stable than the sulfuric acid esters, it is believed that the decomposition of nitrates may be initiated to some extent at least by the small amount of the sulfates or phosphates present.

A critical comparison of the starch with the nitrated starch gave indication of two types of both starch and of nitrated starch; the bright hull which is termed amylopectin nitrate by these workers, and the dark nucleus which they called amylose nitrate. These products may be separated by grinding up the aqueous suspension and then permitting fractional sedimentation to take place. The denser fraction, amylose nitrate, has the higher nitrogen content. The amylose nitrate dissolves in acetone to give a clear solution, whereas the amylopectin nitrate tends to form a slimy suspension.

5. Phosphoric and Sulfuric Acid Derivatives of Starch. Blondeau, in 1843, treated starch with concentrated sulfuric acid and isolated and analyzed two lead salts of the derivative (16). He concluded that the reaction gives two starch bisulfates, or as he termed them, starch sulfonic acids. In 1845, Fehling prepared and analyzed a number of salts of starch bisulfate (48). In the same year Kalinowsky prepared and analyzed the calcium salt of starch bisulfate (76).

In 1883, Liechti reported the preparation of the sulfuric-oleic acid derivative of both dextrin and starch (88). He described the products as being water-soluble, as forming metallic salts, and as hydrolyzing to yield the corresponding carbohydrate and oleic and sulfuric acids.

Hoening, in 1885, reported the treatment of starch with concentrated sulfuric acid to give a derivative which he isolated and described as the barium, calcium, and lead salts (71). In the following year he published another paper on the reaction of concentrated sulfuric acid on starch, and at this time gave some consideration to the structure of the starch molecule (70).

Starch suspended in chloroform was treated with phosphorus oxychloride in the presence of calcium carbonate by Kerb in 1919 (81). He isolated and identified both a calcium and a lead salt of a phosphoric acid ester of a degraded starch.

Vacuum-dried starch was added slowly to chlorosulfonic acid in a mixture of chloroform and pyridine by Tamba in 1923 (149). The product was isolated as its potassium salt and characterized as the salt of an amylodisulfuric acid. This derivative showed no reducing action and gave no coloration with iodine.

Samec, in 1927, treated phosphorus-free amylose with phosphorus oxychloride in pyridine (131). He obtained a phosphorylated derivative which he believed to be an amylopectin.

The first patent to cover derivatives of this general type was obtained by Traube in 1928 (155).

The treatment of alkali starch with *p*-toluenesulfonic acid was effected by Fukushima and Takamatsu in 1929 (56). This derivative was thought to contain 1 acid residue per glucose unit.

6. Carbon Disulfide Derivatives of Starch. Cross, in 1907, saturated dry starch with carbon disulfide, then stirred in powdered sodium hydroxide, dissolved the product in water, acidified the mixture, and added iodine solution to precipitate a starch dixanthate (31).

In 1911, Ost treated starch with a 5 to 20% solution of sodium hydroxide, then with carbon disulfide, and allowed the mixture to stand for 24 hrs. (106). The aged mixture was poured into alcohol to precipitate the sodium starch xanthate. Analysis indicated 2 equivalents of sodium and 2 equivalents of sulfur per glucose unit. Ost observed, furthermore, that this sodium starch xanthate undergoes a progressive degradation with an accompanying decrease in viscosity. He reported, however, that the rate of degradation is somewhat slower than in the case of cellulose xanthate.

A patent was secured by Stern in 1922 to cover the production of the starch xanthates for use as an adhesive for wood veneers (146). According to his procedure, 15 kg. of sodium hydroxide are dissolved in 75 kg. of water and added slowly with agitation to 200 kg. of starch which has been thoroughly stirred with an equal volume of water at 60° C. Into the resulting mixture are stirred 7.5 kg. of carbon disulfide, accompanied by external cooling.

A study on the preparation and properties of the starch xanthates was reported by Wolfenstein and Oeser in 1925 (162).

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CHAPTER XI

OXIDATION OF STARCH

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1. Introduction. The subject of starch oxidation is generally approached from two distinct view-points, that of the research chemist interested in the chemical constitution of starch, who uses oxidative techniques as keys to the intricacies of the starch molecule, and that of the industrial starch chemist who uses oxidative methods to modify starches for certain specific industrial uses. In both view-points there is a common interest in that the same starting material is used and a multiplicity of oxidizing agents is allowed to act under varying conditions. The difference lies in the fact that those interested in studies of the structure degrade the original starch to chemical entities of determinable molecular structure, while the industrial starch chemist is interested in relatively mild oxidative changes that will adapt starch products to special commercial uses. Both view-points deal with the same reaction or series of reactions, the first group of investigators being interested in the complete reaction and in the reaction products, while the second group is primarily interested in those phases of the reaction which apply in the manufacture of oxidized starch products.

The greater portion of the published work on the oxidation of starch comes from academic research laboratories primarily interested in the complete elucidation of the structure of the starch molecule, and as a result their extensive oxidative treatments yield relatively simple chemical compounds. Information dealing with the commercial oxidation of starch must, in general, be obtained from the patent literature. Such sources of information yield only a very limited knowledge of the chemical changes taking place in commercial oxidations. The science has not as yet caught up with the art. Recently, however, a greater scientific emphasis has been placed on studies dealing with starches oxidized under conditions comparable to commercial practices.

In addition to the true oxidation of starch, there are various chemical and biological oxidations which produce such compounds as gluconic, glucuronic, saccharic, citric, lactic, acetic, propionic, and butyric acids, carbon dioxide, acetone, etc., from starch. Because all such oxidations probably proceed through glucose, they are really oxidative products of glucose. Such oxidations are therefore considered to be beyond the scope of this discussion.

2. Historical. The literature on the oxidation of starch dates from Justus Liebig's observation in 1829 (1) that starch is slightly affected by prolonged action of chlorine or chlorous acid. Pelouze (2) in 1838 established oxalic acid

as one of the products formed during the oxidation of starch with concentrated nitric acid. Less extensive oxidation by bromine water and silver oxide allowed Habermann in 1874 (3) to isolate and identify gluconic acid as a degradation product of starch. These early investigations were followed by studies on the oxidation of starch by calcium and potassium permanganate, potassium dichromate, hydrogen peroxide, hydrogen peroxide and ferric chloride, sodium peroxide, fluorine, manganese dioxide, perborates, persulfates, hypochlorites, oxygen, sodium *p*-toluenesulfonchloramide (Activin), etc. For extensive bibliographies of references dealing with the oxidation of starch by various chemicals see Walton (4) and Radley (5).

3. Oxidation by Hydrogen Peroxide. The early investigators began a rather extensive investigation of the use of H_2O_2 as a reagent for the oxidation of starch. Wurster in 1889 (6) reported that H_2O_2 at ordinary temperatures did not attack starch. By making the H_2O_2 solutions slightly alkaline with NH_3 and boiling, it was found that the starch liquefied, the liquefaction being accompanied by the evolution of CO_2 and O_2 (7). Soon thereafter, Syniewski (8) found that a soluble starch could be prepared by the action of Na_2O_2 . The use of acids, alkalis, and salts as catalytic agents for the oxidation of starch by H_2O_2 was suggested by Fernbach and Wolff (9). A series of investigations on the catalysis of the action of peroxide on starch followed this report. Durieux reported (10) that H_2O_2 or FeCl_3 acting alone on starch had very little effect, but that a mixture of the two caused a marked saccharification. This catalytic effect of traces of FeCl_3 or FeCl_2 on the oxidation of starch by H_2O_2 was again substantiated by Dhar (11). Palit and Dhar (12-14) then extended the investigations of the catalytic effect of traces of metal ions to the oxidation of starch and other carbohydrates in alkaline solutions by the action of atmospheric oxygen. Their investigations demonstrate that starch is oxidized to CO_2 and H_2O by bubbling air through an alkaline suspension of the starch in which a small amount of $\text{Fe}(\text{OH})_2$ is suspended. In the presence of sunlight, ZnO was found to act as a photosensitizer promoting the oxidation of starch and other carbohydrates (15). The work of Brown (16) indicates that the action of H_2O_2 on starch in the presence of ferrous ions does not result in an oxidation of the starch, but rather in the hydrolysis of the starch to dextrins and sugars, these products then being oxidized by the peroxide. The hydrolytic stage of the reaction is thus analogous to the amylolytic hydrolysis of starch by enzymes, differing only in that the products are further hydrolyzed and oxidized to give acids and aldehydes of low molecular weight. Brown suggests that the reaction is a true catalysis, the iron acting to transfer energy from the breakdown of H_2O_2 to the starch molecule, the activated starch molecule thus becoming reactive and easily decomposed.

4. Oxidation with Halogens. Oxidation of starch with halogens appears to give four different types of reactions.

1. Oxidation of Aldehyde Groups to Carboxyl Groups—On the basis of the known behavior of starch degradation products under similar conditions it seems logical to believe that oxidation of aldehyde does occur in starch. The

oxidation of aldohexoses to the corresponding acids by the action of the alkaline salts of hypo- halogen acids is the basis of the Willstätter-Schudel titration. The quantitative aspects of this oxidation have been widely utilized, one of the more recent being the use of hypiodite in methanol to oxidize aldomonosaccharides to the corresponding aldonic acids for characterization as benzimidazole derivatives (17). The same fundamental reaction has been utilized for determining the chain length of dextrans produced by the action of α -amylase (18) and of hydrochloric acid on corn starch (19). In both cases the dextrans were quantitatively oxidized by alkaline hypiodite, and the potassium salts of the dextrinic acids were isolated and analyzed for potassium content. Chain lengths calculated from the iodine consumed in the oxidation and the potassium content of the resulting salt were in excellent agreement. Samec (20, 21), Rassow and Lobenstein (22), and Samec and Blinc (23) concluded that the acid groups formed during mild oxidation of starch are derived from the aldehyde groups. The more recent work of Felton, Farley, and Hixon (24) on the oxidation of starch with Br_2 in the presence of excess CaCO_3 indicates that non-uronic acids are formed.

2. *Oxidation of Primary Alcohol Groups to Carboxyl Groups*—Syniewski (25) isolated an "amylodextrinic acid" by the oxidation of amyloextrin with bromine. This product contained glucuronic acid, since the material gave a positive naphthoresorcinol test and yielded about 5.25% furfural upon distillation with HCl (this corresponds to 34.5% glucuronic acid anhydride). More recent studies (24) indicate that as much as 50% of the bromine-oxidized starch may consist of glucuronic acid anhydride. The first isolation of glucuronic acid from a starch product was reported by Farley and Hixon (26). The cinchonine salt of the acid was crystallized from samples of the oxidized starch hydrolyzed by sulfuric acid. Several recrystallizations gave the pure cinchonine salt of glucuronic acid, m.p. 199–200° C.

3. *Oxidation of Secondary Alcohol Groups to Ketone Groups*—Everett and Sheppard (27) have made quantitative studies on the oxidation of more than fifty carbohydrates by bromine in acid solutions. Their data on the analyses of the oxidation products of starch indicated that considerable oxidation occurs on the secondary alcohol group. The presence of considerable reducing power against Fehling's solution at certain stages of oxidation is also indicative of ketone formation (24). Further evidence of the oxidation of secondary alcohol groups to ketones was shown by the formation of an oxime of oxidized starch (26). The nitrogen content of the oxime was found to be equivalent to one ketone group in 65 to 75% of the glucose anhydride units.

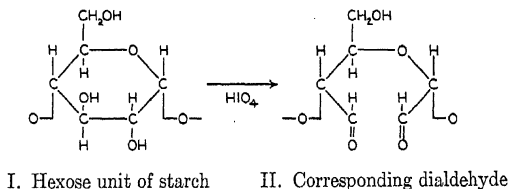
4. *Oxidation of Glycol Groups to Carboxyl Groups*—The oxidation of the glycol group is of the same type as the specific oxidative action of periodic acid. The hydroxyl groups on carbon atoms 2 and 3 in the anhydroglucose nucleus function as a glycol unit. This group is first attacked by the oxidizing agent to convert the hydroxyl groups to aldehyde units, the carbon chain in the pyranose ring being ruptured between carbon atoms 2 and 3. Continued action of the oxidizing

agent then oxidizes the aldehyde groups to the corresponding carboxylic acid groups. That such an oxidation occurs in starch is indicated by the decrease in optical rotation and the increase in calcium content of the acid salts formed by oxidation with bromine. Such a degradation is further supported by the separation of barium salts of dibasic acids containing less than 6 carbon atoms (26).

It should be recognized that all of these four types of oxidation may occur simultaneously during the oxidation of starch by halogens. The halogens appear to lack the specificity required for any one type of oxidation in such a polyfunctional molecule as the anhydroglucose unit.

5. Oxidation by Periodic Acid. In contrast to the more or less random oxidation by the halogens in general, oxidation by periodic acid is characterized by a high degree of specificity. The use of periodic acid for the oxidation of simple sugars was first used by Malaprade (28) in a study of the ring structure of various methyl aldohexosides. Further use of the same oxidizing agent was made by Karrer and Pfahler (29) and Herissey, Fleury, and Joly (30) in studies of the heterocyclic ring structure of hexoses and the alkyl hexosides. On the basis of the experimental evidence obtained in these investigations, Herissey and coauthors suggested that the hexose molecule is oxidized to a dialdehyde with the formation of 1 molecule of formaldehyde. Soon thereafter Jackson and Hudson published their investigations on the determination of the ring structure and α and β configuration of glycosides (31, 32). Oxidation of four of the eight possible methyl- α -*D*-aldohexopyranosides by barium hypobromite yielded the same dialdehyde and the same crystalline strontium salt. This isolation of the dialdehyde substantiated the theory of Herissey, Fleury, and Joly (30) as to the mechanism of the oxidation of hexoses.

Since starch consists of hexose units bound together in some chemical pattern, it would seem logical to apply the method of oxidation by periodic acid to starch. If we assume that starch molecules consist of hexose units having the generally accepted structures, then oxidation by periodic acid should yield substances similar to those obtained from oxidation of the simple hexose sugars.



The first report of experimental oxidations of corn starch by periodic acid is that of Jackson and Hudson (33). The oxidation is carried out by suspending ungelatinized corn starch in an excess of 0.58 *M* aqueous HIO_4 solution and allowing the reaction to proceed at room temperature. At the end of 24 hrs. the quantity of oxidant consumed closely approximates one molecular equivalent, which is the theoretical amount required to oxidize the hexose unit of starch (I)

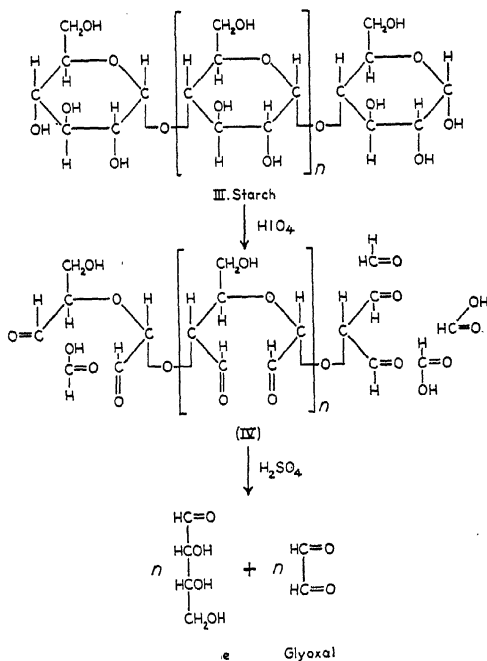
to the corresponding dialdehyde (II). Since the consumption of HIO_4 proceeds at a greatly diminished rate after the consumption of 1 mole of oxidizing agent, it is apparent that the principal reaction is completed at this stage. This treatment of corn starch results in a quantitative yield of the oxidized product, the appearance of which is much like that of the parent corn starch when viewed under the ordinary microscope. However, when viewed between crossed nicols, the oxidized granules show a uniformly dark field in contrast to the characteristic cross of untreated starch granules. This loss of birefringence in the granules is readily followed by placing a few granules of corn starch in a drop of approximately 0.5 *M* HIO_4 and observing them between crossed nicols. The dark bands of the polarization crosses gradually spread and within a few minutes completely disappear.

The product obtained by the mole-for-mole periodic acid oxidation of corn starch is insoluble in cold water, soluble in hot water, gives no color reaction with iodine, reduces Fehling's solution, forms an amorphous precipitate with phenylhydrazine at 25° C., is not attacked by malt diastase, and has a specific rotation of about +9° at 20° C. in sodium light. If a thin layer of an aqueous solution of the oxystarch is poured onto a glass plate and allowed to dry, a clear transparent film can be produced. Hydrolysis by boiling 0.1 *N* aqueous HCl gives a solution with a levo equilibrium rotation of -13° to -16°. Because this rotation approximates that reported for *d*-erythrose, and because hydrolysis of the proposed dialdehyde structure would be expected to yield *d*-erythrose, Jackson and Hudson suggested that the main optically active component of oxystarch hydrolysates might be *d*-erythrose.

Working concurrently, but independently, Caldwell and Hixon (34) and Jackson and Hudson (35) carried on further investigations relative to the oxidation of starch by periodic acid. These investigations have materially assisted in the elucidation of the mechanism of oxidation by periodic acid. On the basis of fairly well accepted concepts, the starch molecule consists of a chain of glucopyranose units joined by 1,4- α -glucoside linkages (36). The theoretical oxidation of such a molecule by periodic acid would proceed as shown in the first two steps of the following reaction. The intermediate glucose units would be oxidized as reported by Jackson and Hudson (33), while the terminal units would give rise to 3 molecules of formic acid (28, 30) and 1 molecule of formaldehyde. Acid hydrolysis of the oxidized starch should yield glyoxal and *d*-erythrose as the principal products and certain other decomposition products in small amounts. This hydrolytic reaction is shown in the final step of the following reaction (cf. p. 229).

By assuming this theoretical oxidation to be correct, it is evident that the quantitative determination of the amount of formaldehyde formed during oxidation of the starch by periodic acid should furnish a method for measuring molecule size. Caldwell and Hixon (34) utilized this method for the measurement of the molecular size of starches and dextrans. By comparing the chain length of the various dextrans as calculated from the experimentally determined

amount of formaldehyde with the chain length of the same fraction as calculated from the reducing value (37), satisfactory correlations were obtained. The analytical data obtained from oxidations by periodic acid therefore suggest that the chain length of the fundamental starch unit is much longer than the 25 glucose units obtained by Haworth, Hirst, and Woolgar (38) from their methylation data.



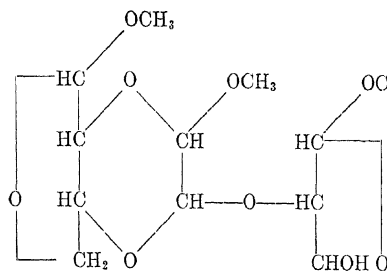
The first characterization of the products obtained by the acid hydrolysis of periodate-oxidized corn starch was the isolation and identification of glyoxal by preparation of the osazone and benzylphenylosazone (34). The osazone crystallizes as light yellow, elongated, hexagonal plates with a melting point of 172–175°; the benzylphenylosazone crystallizes as fine white needles melting sharply at 195°. This isolation of glyoxal serves to substantiate the theoretical equation for the oxidation of the non-terminal glucose units of starch by periodic acid as written above. Identification of *d*-erythrose, the remaining hydrolytic product in the proposed mechanism, would furnish conclusive proof for the rupture of the non-terminal glucose units between carbon atoms 2 and 3. Caldwell and Hixon were unable to identify *d*-erythrose in the hydrolytic products. However, they report that a private communication from Jackson and Hudson informed them that, in addition to glyoxal, *d*-erythrose has been identified in the hydrolytic cleavage products of periodic acid oxystarch (34). The presence of glyoxal interferes with the isolation and identification of *d*-erythrose. By treating the

hydrolysis products of periodic acid oxystarch with bromine water, the glyoxal can be removed as an interfering reactant by oxidation to oxalic acid and precipitation as the barium salt. The *d*-erythronic acid is then crystallized as brucine *d*-erythronate, m.p. 211° (with decomposition). *d*-Erythronic lactone, m.p. 104–105°, can then be prepared from the pure brucine *d*-erythronate (35). These results prove that periodic acid breaks the carbon chain of the non-terminal glucose units of the starch molecule between carbon atoms 2 and 3. They also offer confirmatory evidence for the generally accepted structure of the predominating units in starch.

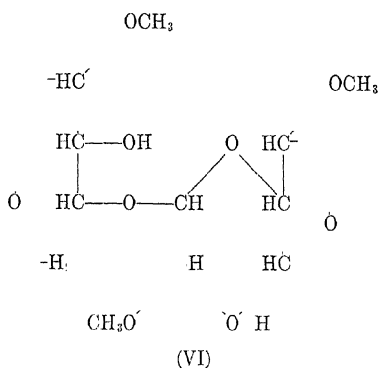
The oxidation of starch by periodic acid gives the same results whether the oxidation is carried out on the raw granules or on gelatinized starch. Jackson and Hudson have always applied the oxidizing agent to the whole starch grains, while Caldwell and Hixon applied periodic acid to gelatinized starch pastes. Their results agree in all respects. When periodic acid was applied to soluble starch and insoluble powdered starch, by the same investigators, the rate curves for periodate ion consumption for both starches were found to be practically superimposable (39). It is thus evident that the periodate ion is capable of penetrating into the starch granule and simultaneously attacking all parts of the micellar structure.

Further studies on periodate-oxidized starches show that the application of methanolysis to the oxystarch yields small quantities of a crystalline substance of unknown composition (40, 41). The material is a white, crystalline, levorotatory compound, melting at 148°, and obtainable in yields up to 2%. It is non-reducing, but after mild acid hydrolysis it reduces Fehling's solution and appears to be the methyl acetal of an aldehyde or ketone. The same crystalline product is obtained from periodate-oxidized corn, wheat, potato, arrowroot, and soluble potato starch.

The excellent research of Michell and Purves (42) has given considerable information as to the nature of this crystalline compound. Their work shows that the compound has the empirical formula $C_{10}H_{12}O_5(OH)(OCH_3)_3$, that all 5 oxygen atoms in the saturated aliphatic nucleus are centers of ether or acetal linkages, and that three cyclic ring systems are probably present. Remembering that the compound is derived from periodate-oxidized starch (II), which consists of repeating dialdehyde units containing 1 erythrose and 1 glyoxal residue, it becomes possible to form two structures which would satisfy the experimental evidence. These structures vary only in the point of attachment of the glycosidic bond. Michell and Purves thus propose that the crystalline substance isolated from methanolysis of periodate-oxidized corn starch consists of a methylerythrofuranoside unit combined in a 1,4-dioxane ring with a glyoxal residue, to which a second methylerythrofuranoside residue and a methyl group are separately attached through glyoxal hemiacetal linkages. Structures (V) and (VI) satisfy these requirements.

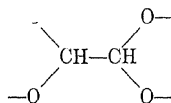


(V)



(VI)

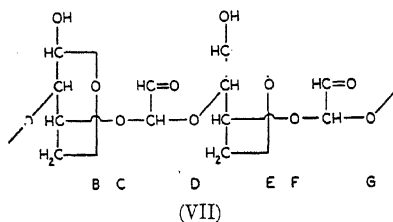
Additional experimental evidence to support the proposed structures is furnished by further methanolysis of the crystalline compound under drastic conditions (42). Steam distillation of the products of methanolysis gives a good yield of glyoxal tetramethylacetal. This provides evidence for the presence of the



unit in the crystals.

The *p*-toluenesulfonyl monoester of the crystalline compound derived from the oxystarch is prepared by the action of *p*-toluenesulfonyl chloride in pyridine. The formation of the monoester supports the presence of only one hydroxyl group. Treatment of this monoester with pure sodium iodide in dry acetone under pressure at 100° C. for 2 hrs., followed by recovery of the products, shows that about 85% of the original *p*-toluenesulfonate is recoverable in a pure crystalline form. Since the *p*-toluenesulfonyl group in derivatives of primary alcohols is known to be replaced quantitatively by iodine under these conditions (43), the recovery of the major portion of the original monoester shows that the crystalline oxystarch derivative does not contain a primary alcohol group.

The formation of such ring systems as those suggested for the methanolysis products of periodate oxystarch are at first difficult to comprehend. Their formation can best be explained by assuming that the erythrose portion of the dialdehyde oxystarch structure follows the normal tendency of erythrose to condense to a furanoid form (44). By assuming that the furanoid ring forms in every erythrose unit, we can write the structure of starch oxidized by 1 mole of periodic acid, as shown in (VII). The glyoxal acetal bonds, A, D, G, etc., are

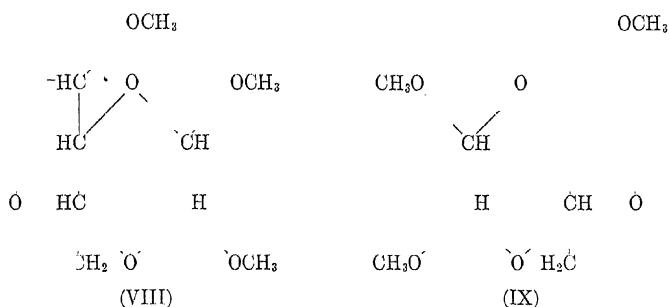


the glycosidic bonds of the original starch; the furanoid bonds, B, E, etc., are formed by the normal cyclization of the erythrose units; and C, F, etc. are the pyranoside rings in the original starch. Methanolysis of such a structure with hydrogen chloride in methanol would cleave the glyoxal acetal bonds and the original pyranoside linkages, but would have no effect on the newly formed furanoside rings. Each cleavage would produce a new hydroxyl group on either the second or third carbon of the erythrose residue, and the condensation of this newly formed hydroxyl group with the free aldehyde group in the adjacent glyoxal unit would result in a substituted 1,4-dioxane ring. The probability of such a hemiacetal condensation is supported by similar cyclizations between known molecules of a similar structure. The formation of (V) can then be explained by assuming that methanolysis first occurs at A, followed by cyclization of the newly formed hydroxyl group on carbon atom 2 of the glyoxal residue, then methylation of the hemiacetal hydroxyl groups, and finally a rupture at F. If the initial hydrolysis had occurred at F and the final methanolysis at A, structure (VI) would be produced. Since bond A is glycosidic in nature and bond G pyranosidic, methanolysis of the respective linkages might not proceed at the same rate. This would give rise to unequal amounts of (V) and (VI).

An additional complicating factor is the fact that formulas (V) and (VI) should each give rise to eight isomeric forms depending on the various possible α and β configurations of the three methoxyl groups. The crystalline material isolated from the methanolysis of the periodate-oxidized starch is believed to be one of these isomers in a pure state. The mother liquor from this crystallization was carefully examined by Michell and Purves (45), and their experimental evidence indicates that the material is structurally identical to the crystalline fraction. This entire fraction must, therefore, consist of two or more isomers of structures (V) and (VI). The structural identity of these two fractions gives a total yield of 22% of these isomeric hexahydro-3, 5-dimethoxy-2-(1-methyl-3, or 2-erythrofuranosyloxy)furo-[3,4]-*p*-dioxins. The exact identification of the

isomeric forms present in the methanolic degradation products of periodate oxystarch will serve as future problems for the research chemist.

Since materials of structure (V) and (VI) account for less than 25% of the total oxidized starch, Michell and Purves (45) have extended their studies to other fractions obtained in the methanolysis of periodate oxystarch. Fractional distillation of the methanolic cleavage products gives a mobile, colorless material, which distills at 116–119° C. at 5 mm. pressure, sp.gr. (20°/20°) of 1.204, $n_D^{20} = 1.4488$, and a molecular weight of 226 in CCl_4 . An ether + petroleum ether solution of the fraction on standing gives a white, bulky precipitate. Recrystallization yields a pure crystalline substance, m.p. 97–98°, $[\alpha]_D^{20} = -59.1^\circ$, and a molecular weight of 212 in CCl_4 . On the basis of their studies Michell and Purves believe the entire fraction to be a mixture of isomeric hexahydro-2,3,5-trimethoxyfuro-[3,4]-*p*-dioxins (VIII) and (IX), one of which crystallizes as

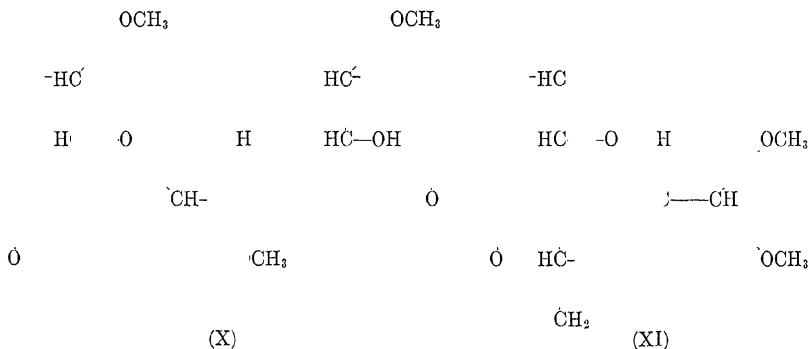


described above. The entire fraction accounts for another 18% of the original oxystarch. The formation of such structures by methanolysis of oxystarch (VII) would require two types of bond rupture. Cleavage of oxystarch at bonds A, D, G, etc., followed by condensation and methylation, would give rise to (VIII), while cleavage at C, F, etc. would give rise to (IX). Structures (VIII) and (IX) can also be derived by removing the erythrose residue from formulas (V) and (VI). This degradation has been demonstrated by Michell and Purves (45). Treatment of a mixture of the isomeric forms of (V) and (VI) with a 10% solution of hydrogen chloride in dry methanol gives a 36% yield of a fraction which has properties identical to those of mixtures of the isomeric forms of (VIII) and (IX). Analytical data for the two fractions give additional proof of their identity.

Continued efforts to identify erythrose in a hydrochloric acid hydrolysate of (V) and (VI) have been unsuccessful. Analytical data suggest that the glyoxal and erythrose which initially form under hydrolytic conditions immediately recombine under the influence of aqueous hydrochloric acid to give unidentified condensation products. That cyclic acetals can be formed under similar conditions is demonstrated by the acetal condensation of bromoacetaldehyde and mannitol (46). A more striking example is the formation of 2,3-ethylenedioxy-

dioxane in 20% yields by the condensation of glycol and glyoxal tetramethylacetal in normal aqueous or aqueous alcoholic hydrogen chloride (47). In the last example, condensation to give a cyclic acetal occurs under conditions which supposedly are ideal for hydrolysis of such compounds. Therefore, the hydrolytic conditions applied to (V) and (VI) in an attempt to form erythrose and glyoxal may be ideal for their primary formation, yet these same conditions may also be ideal for their recondensation into other cyclic acetals. This would explain the failure to isolate erythrose as a hydrolytic product.

The possibility of recondensation of hydrolytic products introduces an added factor in the study of the methanol + hydrogen chloride breakdown products of periodate oxystarch. If we assume that methanolysis should break every bond in (VII), we would have a mixture of erythrose and glyoxal, or their methyl derivatives. If a reversion process then set in, structures (V), (VI), (VIII), and (IX) could be formed by condensation of the glyoxal and erythrose. Under these conditions the products of the methanolysis might not have the 1,4-dioxane structures tentatively assigned to them but, rather, be cyclic acetals of structures (X) and (XI). It seems improbable that structures (V), (VI), (VIII), and (IX)



could be produced in the observed yields by reversion processes. However, as pointed out by Michell and Purves (45), the possibility of a recondensation of the initial scission products must be simultaneously considered along with cyclization of carbonyl with conveniently situated hydroxyl groups as an explanation of the formation of the methanolic degradation products of periodate oxystarch.

The quantitative determination of the glyoxal units present in oxidized starches serves as a means for the determination of the dialdehyde type of oxidation in periodate oxystarches (39). Methanolysis with 10% hydrogen chloride in methanol yields glyoxal tetramethylacetal and the 1,4-dioxane derivatives discussed previously. Simple steam distillation of the products of samples of known composition, followed by determination of the distilled glyoxal by precipitation with 2,4-dinitrophenylhydrazine, shows that only about one-half of the expected glyoxal residues is distilled. Studies on the distillation rates of

the products known to be present have shown that the cyclic 1,4-dioxane derivatives do not distil under ordinary steam distillation techniques. However, if the aqueous methanolysis products are steam-distilled nearly to dryness, additional water added, and steam distillation continued until the still pot is again nearly dry, over 90% of the theoretical glyoxal residues is steam-distilled. This method has been carefully studied by Grangaard, Gladding, and Purves (39) and, under the conditions which they have established, at least 90% of the glyoxal units theoretically present in periodate oxystarch can be recovered. This gives us an analytical tool for the study of starch oxidations in which dialdehyde structures are formed. The analytical method is also applicable to oxidations of cellulose.

6. Oxidation by Commercial Methods. The published literature contains little information on the changes brought about by the commercial oxidation of starch. The closest approximation is given by Farley and Hixon in their report on the properties of starch oxidized by hypochlorite produced by the electrolysis of alkaline sodium chloride solutions (26). The changes during oxidation were followed by determinations of hot viscosity, rigidity, gel strength, reducing power, turbidity, volume of swollen granules, quantitative birefringence, digestibility by β -amylase, and microscopic observation of the swollen granules. Rigidity, gel strength, and turbidity decrease proportionally to the amount of oxidizing agent used. With low hypochlorite consumption no increase in reducing power is observed, but the more highly oxidized products show marked increases in reducing power. Oxidation decreases the amount of maltose produced by the action of soybean β -amylase. Photomicrographs of the starch granules oxidized with various quantities of hypochlorite and gelatinized in 0.70% NaOH show marked differences. With increased oxidation the granules become more resistant to swelling, until starch granules oxidized with 0.5 equivalent of chlorine per unit fail to swell. These highly oxidized granules start to disintegrate along the visible radial fissures, causing the granule to break into several fragments. These fragments then break into numerous small pieces and finally dissolve in the solution of sodium hydroxide. It is most interesting to note that this highly oxidized starch upon centrifuging gave an almost clear paste of very low viscosity and no sediment of swollen granules.

The experimental observation that the quantitative birefringence of starch granules does not change by oxidation with as much as 0.5 equivalent of chlorine per glucose unit suggests that the crystalline regions of the starch granule are not attacked by the oxidizing agent (26). However, swelling experiments show that these oxidizing agents penetrate deeply into the starch granules, causing marked changes in the rate and extent of granule swelling. On the basis of these experiments Farley and Hixon suggest that the oxidizing agent attacks the starch granules between radial starch crystallites. Solution of the oxidized portions would give rise to the cracks and fissures observed in oxidized starch granules. Upon continued reaction the oxidizing agent would continue to attack the non-crystalline portion of the starch, and thus penetrate the entire granule. Such a mechanism of attack would explain the partial solubilization of

starch upon oxidation, the formation of cracks and fissures, and the characteristic "swelling" behavior of oxidized starch; further, this method of attack would allow the starch granule to retain its quantitative birefringence.

Commercially oxidized starch resembles raw starch in that it retains practically the same granule structure, shows typical polarization crosses, is insoluble in cold water, and shows the characteristic starch color reactions with iodine. However, when heated with water the unoxidized starch yields pastes or gels, while the oxidized starches at equal concentrations give thinner bodied solutions, the differences varying with the degree of oxidation. Because of the increased fluidity of the pastes the oxidized starches are commonly designated as "thin boiling starches." This term is also applied to acid-modified starches.

The degree of modification of oxidized starches is expressed in terms of fluidity. Fluidity refers to the volume of a suspension of pasted starch of specified concentration which will flow through a standardized orifice in a specified period of time as compared to a specific volume of water which has flowed through the same orifice in the same length of time. For example, a "60 fluidity" starch means that 60 cc. of the starch suspension (the concentration of the suspension varies with the type of starch used) have flowed from the standard orifice in the same period of time that 100 cc. of water would require.

Oxidized starch has (1) a shorter cooking time, (2) higher fluidity, (3) increased adhesiveness, (4) lower rate of congealing, and (5) gives a less turbid suspension than the parent starch. Films formed by drying oxidized starch pastes are of a tough and horny character in comparison with the extremely brittle films of unoxidized starch. These properties make oxidized starch particularly adaptable to the surface sizing of paper and to the sizing and finishing of textiles.

Raw cotton yarns, containing no size, do not have sufficient strength to withstand the stretch to which they are subjected on the loom if used as warps, and do not offer the proper resistance to abrasion. By sizing the warp threads with a proper mixture of starch, gums, lubricants, and softeners the yarn is conditioned to have the necessary stiffness, strength, elasticity, and pliability to withstand the mechanical wear and strain of weaving. The greater adhesiveness of the oxidized starches gives warps with individual fibers properly cemented together so as to form a strong, elastic, pliable thread with the proper amount of starch on the surface to protect the yarn from abrasive action. These oxidized starches cook without foaming in the kettle, require less gum and compound, flow through the pipe lines freely, remain at a constant fluidity with continued circulation, and drain out of the size kettle, storage tanks, and size box without leaving any hard size. In addition, the oxidized starches are exceptionally white and clean.

In paper making, starches and the highly oxidized starch gums are used to close the pores of the paper, to lay fuzz on the surface, to increase tensile, fold, and bursting strength (Mullen), and to give "feel" and rattle to the paper. The starch gums also act as protective colloids resulting in a finer, more uniform dispersion of the rosin size.

The most common agents used commercially for the oxidation of starch are sodium and calcium hypochlorite. For this reason many commercially oxidized starches have been referred to as "chlorinated starches." This is a misnomer, because the chlorine does not substitute into the starch molecule as the name infers, but rather serves to provide the oxidizing potential. This oxidation is brought about by the reduction of the positive valent chlorine in NaOCl to the negative valent chlorine in NaCl, with the subsequent release of $\frac{1}{2}$ mole of oxygen.

The general commercial method for producing hypochlorite-oxidized starches consists of treating aqueous starch suspensions (about 36%) with a hypochlorite solution (6 to 8% active chlorine) containing a slight excess of alkali. The hypochlorite solution is added in small portions so that the heat generated can be dissipated by the cooling system, the general practice being to keep the temperature between 90° and 125° F. The heat produced is probably due to three exothermic reactions: (1) the heat of adsorption, (2) the heat of decomposition of the hypochlorite, and (3) the heat generated in oxidizing the starch (22). When the reaction is judged to be approaching the desired degree of oxidation, a sample of the starch milk is removed, filtered, and washed on a vacuum filter, and the fluidity of a paste of definite concentration prepared from the cake is then determined. When a product of the desired fluidity is obtained the oxidation is stopped by adding an antichlor (usually sodium bisulfite), and the slurry is adjusted to the desired pH (usually 3 to 7), filtered, washed, water removed to about a 47 to 50% moisture content, and dried. By varying the quantity of hypochlorite used and the time, temperature, and pH of the reaction, an entire series of oxidized starches can be produced.

In addition to the wet process for producing hypochlorite-oxidized starch, some use is made of a dry process. In this process completely dried starch is put into a mixer provided with a water jacket. A solution of sodium hypochlorite containing 8% of available chlorine and 2% of sodium hydroxide is sprayed on the continuously agitated starch. The rate of addition of hypochlorite and the amount of cooling water flowing through the jacket are adjusted so that the maximum temperature is maintained at 45° C. (113° F.). At the end of the reaction the product usually contains about 32% moisture, which is then reduced in any suitable drier to 10 to 20% (48, 49).

Nearly all commercially oxidized starches are thinner boiling and have less tendency to congeal than the parent starches. However, it is possible to prepare starches with increased fluidity by treating with hypohalites under properly controlled conditions. For example, Bryant (50) describes a process for increasing the viscosity of starch products in which very small amounts of sodium hypochlorite are added to the starch slurry before the final removal of water. The amount of hypochlorite added is just slightly in excess of that amount calculated to oxidize the sulfur dioxide present in the starch. Thus, the function of the oxidizing agent appears to be the removal of the sulfur dioxide, which may act as a modifying agent in the subsequent pasting operation.

The oxidation of starch by halogens in slightly acid solutions to produce starches with maximum hot paste viscosity and low cold paste viscosity has been patented by Kerr (51). This patent is based upon the discovery that when starch is treated with a halogen, in properly regulated amounts and at temperatures of about 125° F., the viscosity curve of the hot pastes rises very sharply during the first part of the reaction and then declines to give typical "thin boiling starch" viscosities. A similar rise in viscosity in the early phases of starch oxidation by electrolytic hypochlorite in alkaline sodium chloride solutions is reported by Farley and Hixon (26). Their work shows that with increasing degrees of oxidation the viscosity of starches increases to a maximum at 0.2 equivalent of chlorine per anhydroglucose unit, and then decreases with additional oxidation.

Another interesting halogen-oxidized starch is described by Kerr (51). By treating a 30% starch slurry with chlorine or bromine (preferably bromine) at 75° F., a thick boiling, thick setting starch can be produced. These thick boiling, thick setting starches are capable of adsorbing more water than the parent starch from which they were prepared. By following this oxidation with an acid treatment, a product can be obtained the paste of which is less congealing than a product of the same fluidity made by hydrolyzing raw corn starch with acid alone.

Oxidation of dry starch by chlorine gas also yields products whose pastes have viscosities lower than those of the parent starch (52). The patents covering this process claim that "by varying the temperature of treatment, the length of the treatment, which may vary from one to twelve hours, and the amount of chlorine used, it is possible to produce any modified starch product ranging from thin boiling starch, substantially insoluble in cold water (1% soluble) to dextrines which have solubilities in cold water of over 90%."

The oxidation with hypochlorite is also utilized in the manufacture of refined sago starch products from crude sago flour (53). From 0.25 to 6.0% of available chlorine, based on the dry weight of the starch, is added to an aqueous suspension containing 25 to 30% of starch. The available chlorine is always added as an alkaline hypochlorite solution, preferably as calcium hypochlorite. After the reaction is allowed to proceed for a specified period of time, the starch milk is screened, washed on continuous filters, resuspended, the pH of the slurry adjusted, and the starch filtered and dried. In this manner a sago starch product pure white in color and substantially free of impurities is obtained. The degree of modification of the finished product is controlled by the extent of the preliminary chlorine treatment.

The other large class of chemical compounds used in the preparation of oxidized starch is the per compounds; *e.g.*, sodium perborate, ammonium persulfate, alkaline peroxides, and alkaline permanganate. The usual practice with perborate and persulfate is to mix about 1% of the reagent with the air-dried starch. A slight physical modification is then brought about when the mixture is suspended in water and cooked to a paste. In other cases the starch paste is

prepared, the oxidizing agent added, and cooking continued until modification is complete. In both methods, with either perborates or persulfates, only moderately modified starches are obtained. Persulfates will also yield modified starches by the wet process (54). The persulfate is added to the starch slurry, the correct amount of acetic or hydrochloric acid added, and the reaction is allowed to proceed at room temperature. The oxidized starch is recovered by filtration, washed thoroughly, and then dried.

As with the perborates and persulfates, alkaline peroxides, especially barium peroxide, can be mixed with the dry starch (55, 56). In the dry state or in aqueous suspensions at room temperatures there is little or no modification. Upon heating the aqueous suspension, pronounced modification occurs, the degree of modification depending on the quantity of peroxide added and the length of time the aqueous suspension is heated. The wet process is also applicable when peroxides are used as the oxidizing agent (57). In this method calcium peroxide is added to the starch slurry, which is heated to 52° C. (126° F.) and maintained at that temperature for about 24 hrs. The oxidation mixture, which has a pH of about 11, is neutralized to pH 7.0 with hydrochloric acid, the water removed by filtration, resuspended in water, filtered and washed to remove the soluble salts, and then dried. Starches prepared in such a manner are thin boiling but thick setting.

The treatment of moist starch with from 0.05 to 0.15% hydrogen peroxide at 100–110° F., followed by drying in a Buell drier, yields starch free from thermophiles (58). No modification of the starch is produced.

Oxidation of starch by alkaline permanganate decreases the viscosity and increases the transparency of the pastes prepared therefrom. The modification can be continued until red-staining iodine reaction products are produced. Most "permanganate starches" have a yellowish tinge due to the retention of small traces of manganese compounds. Treatment with such agents as sulfur dioxide, sodium hydrosulfite, or oxalic acid destroys the residual color.

Sodium *p*-toluenesulfoncholoramide, commonly known as chloramine-T or Activin is reported to be widely used for the commercial solubilization of starch in Europe. The chemical is said to solubilize the starch without any formation of dextrins or sugars (59).

The production of such a wide variety of oxidized starches to meet the specific requirements of the trade demonstrates the extent to which the art of starch oxidation has progressed. Practically nothing is known of the chemical changes which the starch molecule, and probably the granule, undergoes in these treatments. The polyfunctional nature of the starch molecule and the complex arrangement of such molecules into the highly organized starch granules make the problem extremely difficult. It is thus evident that the elucidation of the chemical and physical phenomena involved in the art of starch oxidation must lie in the results of future research investigations.

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CHAPTER XII

DEXTRINIZATION

G. V. CAESAR

In this chapter the possible mechanisms of the degradation of starch in the dry state by the application of heat and chemicals in varying quantities are discussed. The subject matter concerns certain physical and chemical properties of commercial dextrans produced from starch by normal methods of dry conversion. The graphical data obtained for these properties form the basis of the theoretical discussion. The chemicals used in the conversions discussed are weak aqueous mineral acids which are applied by the usual method of spraying them on normally dried starch and subsequently subjecting the acidified starch to heat at different temperatures and for varying periods of time. Starch degradation products of unusual character or manufacture are not described. Discussion is confined primarily to the theoretical aspects of the commercial types of starch degradation products known to the trade as white dextrans, canary dextrans, and British gums. Additional, technical aspects of the subject will be discussed in Chapter XIII.

1. White Dextrans. In Fig. 77, some of the more important indices of dextrinization, *viz.* viscosity, alkali lability (1), and solubility, are plotted against time of conversion for a white corn dextrin in the low to medium range of solubility (in water at 25° C.).

The temperature range of the conversion of starch to white dextrin is relatively low, and the proportion of aqueous acids incorporated with the starch is relatively high. The effect of the aqueous acid is most strikingly revealed by the high rate of decrease in viscosity. Owing to rapid congealing, combined with the high magnitude of viscosity values, it was impracticable accurately to determine kinematic viscosity values at conversion times less than about 45 min. The drop in the viscosity in the early stages of conversion is obviously tremendous. Near the beginning of the conversion it can be demonstrated best, perhaps, by

special methods for testing thick consistences (2, 3). The tremendous rate of change in the early stages of conversion is clearly indicated in Fig. 77. The rate is still relatively high, even at the end of the conversion period. A somewhat similar effect is observed in the curve for percentage of solubility, although it appears that the rate of change in respect to solubility (slope of curve) is more constant. An accurate determination of solubilities below a value of 1% is difficult, but it is probable that the rate of formation of soluble substance is high at all stages of the conversion and relatively uniform, irrespective of the magnitude of the changes. Semilogarithm plots are of great assistance in showing these differences. The alkali lability values shown in Fig. 77 lie on a smooth curve, the slope of which slowly diminishes from a value of 23, for the unmodified corn starch, to 58 for the converted dextrin. Alkali lability is obviously a less sensitive index, in this particular conversion at least, than is either viscosity or solubility.

Let us consider the significance of these curves in respect to changes in the inner structure of the starch. Starch degradations involving hydrolytic scission of the glucosidic linkages are discussed elsewhere in this volume. From a microscopic examination, the behavior in water of thin boiling starches and dextrans, particularly the white dextrans, suggests that this action, when involved in dextrinization, is highly specific. It appears that the framework of the granules has been profoundly shattered. They swell only a little, split into fragments, and show more or less solution or sloughing away of surface layers. The highly converted, soluble dextrans (white and canary) exhibit little or no preliminary swelling. The microscopic picture is highly suggestive of the snipping action of an infinitesimal pair of scissors on the molecular clusters of molecules which are packed into the granular packages. The "scissors" are $[H_3O]^+$ ions and their place of action is the 1-4 O' linkage, in unbranched or so called linear chains, and the 1-6 O' linkage in branched chains. An examination of Fisher-Hirschfelder atomic models suggests that the 1-6 links should be more susceptible to scission than the more occluded 1-4 links. In this connection, Kerr (4) remarks that "acid-converted starches produce a higher yield of butanol precipitate, which is very unstable, than is produced from original starch. This action of acid is explained as the production of new short linear chains by breaking-off the branches from the complexly constituted amylopectin types." This result is precisely what might be expected from spatial configurations, provided that the models represent with reasonable accuracy the stereoconfiguration.

Let us assume, accordingly, that the initial breakdown or hydrolysis of starch is more pronounced at 1-6 linkages than at 1-4, and that subsequent scission is more or less evenly distributed. The viscosity of aqueous dispersions of the dextrin at a given concentration and temperature (in the case of Fig. 77, 20% and 130° F.)¹ is then a function of chain length distribution of the snipped polymers and the structure in the liquid of the associations of these polymers

¹ The dextrin was first cooked thoroughly at 180° F. and then cooled to 130° F.

probably formed through hydrogen bonding.² If branched chains represent the structure of the starch component of high viscosity (4), as indeed they should, the extremely high rate of decrease in viscosity in the early stages of conversion is explained by scission of 1-6 linkages. The rate of change, later, might be expected to decrease as hydrolytic degradation shifts more and more to the narrower unbranched component. In all dextrinizations promoted by aqueous acids, the plots of the log of viscosity as a function of time are of the general form of Fig. 77. Viscosity, when it may be determined accurately,³ is one of the most sensitive and significant indices of starch degradation.

The solubility curve needs little explanation beyond what has already been said. The solubility (at 25° C.) would be expected to be low until enough of the chains are sufficiently depolymerized to form a fairly stable dispersion or "solution" in water at room temperatures. It is a common and useful index of degradation and has commercial utility for various purposes.

Interpretation of data for the lability to alkali is more difficult and uncertain. Taylor's method (1) is the one heretofore employed by the author. Schoch (5) uses a modification for which he claims advantages. Taylor's technique is difficult and complicated, and reproducible results are possible only with ample experience. Exactly 50 mg. of sample are digested for 1 hr. in 0.1 *N* NaOH in a boiling water bath. A color change ensues and the intensity of the brown tint is roughly proportional to the degradation suffered by the starch in dextrinization. Excess iodine is added, and the solution is allowed to stand in the dark for a specified time. The milligrams of iodine absorbed per 100 mg. of sample are then determined. Maximum iodine absorption occurs for the white dextrins. During the preparation of the canary types, in which process heat plays an important rôle, the curve for alkali lability passes through a definite peak and then declines, often to a very considerable extent, a phenomenon also present but less marked in conversions of modified British gums. Alkali lability is probably associated with chain degradation, state of aggregation of micellar complexes, and chemical modifications, but its chemistry is still obscure. It is an interesting and useful adjunct to other quantitative indices of dextrinization.

The conversion of starch to white dextrins is thus essentially a degradation or depolymerization process probably beginning with branched chains and extending to unbranched chains. The purpose of the heating at low temperature is to increase the activity of $[H_3O]^+$ ions, the primary reagent.

Fig. 78 shows the fundamental viscosity-temperature relationship of three white corn dextrins ranging in solubility from about 3% to 20% (at 25° C.).⁴ It is quite apparent that Curves 1 and 2 represent products of similar type or "family," but that Curve 3 is more distantly related. The rate of the change in viscosity with rising temperature increases smoothly from 70° F. (294.2° K.)

² See Chapter IX.

³ Viscosity data of Fig. 77, etc., were obtained in modified Ostwald viscosimeters.

⁴ Data are plotted on semilogarithmic paper against the reciprocal of absolute temperature, for reasons described in Chapter IX.

to 176° F. (353.1° K.), for both Curves 1 and 2; for Curve 3 the rate of drop is more uniform, a common characteristic of homogeneous liquids. The downward trend of Curves 1 and 2 probably is indicative of a continuously increasing rate of breaking of hydrogen bonds with increasing temperature. It is suggestive of relative heterogeneity of colloidal structure. The concentration of the dextrin must be considered in making comparisons of this nature. The plot of the logarithm of viscosity *versus* $1/T$ may indicate homogeneous liquid structure at a 10% concentration but at 20% may indicate quite the reverse. True homogeneity of structure is indicated when the curve for the logarithm of viscosity *versus* $1/T$ takes the general form of Curve 3. The lower portion of the viscosity curve of Fig. 77 represents approximately the stage of conversion of the dextrans of Curves 1 and 2 of Fig. 78. The form of the viscosity-temperature curves of these dextrans is proof of their heterogeneity.

2. British Gums. These are produced essentially by a heat treatment of starch, although for types which might perhaps be termed "modified British" a small amount of aqueous acid may be employed to modify the more or less heavy bodied starch characteristics of ordinary British gums. Although suitable data for plotting viscosity *versus* time of conversion are not available, it may be stated that for British gums the slope of the viscosity curve, as a function of

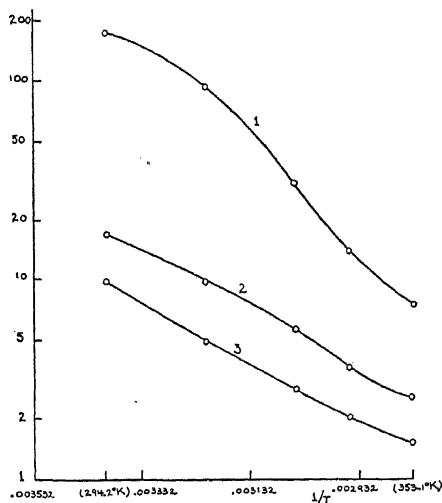


FIG. 79. Viscosity (centistokes) *versus* $1/T$ for Curve 1, British gum, heat-converted, 20% soluble; Curve 2, British gum, heat- and acid-converted, 90% soluble; Curve 3, canary, British gum, heat- and acid-converted, 95% soluble.

time of conversion, is much less than for conversions of white dextrin, as is also the magnitude of the change in alkali lability (6). There is some evidence that the mechanism of heat conversion includes dissociation of primary valence chains through the breaking of hydrogen bonds, dehydration to form inner ether linkages,

and possible oxidation effects, particularly at high temperatures. The whole picture is extremely complex, much more so than for white dextrins, and will be further discussed under the heading, "Canary dextrins."

The viscous properties of British gums and a modified type are shown in Figs. 79 and 80. They are of interest in reflecting structural conditions. Curve 1, Fig. 79, represents a heat-converted corn starch product with about 20% of soluble material; Curve 2 is a type of modified British gum produced with the

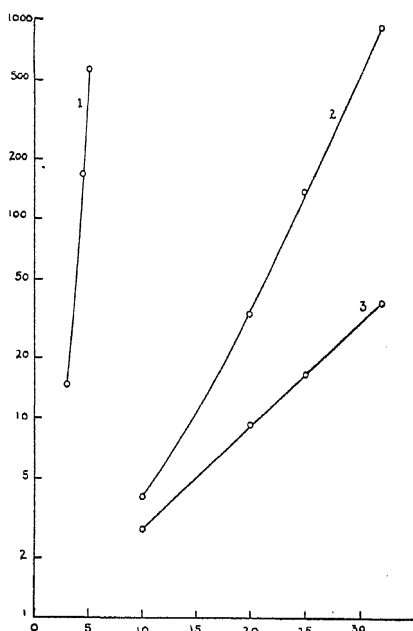


FIG. 80. Viscosity (centistokes) *versus* per cent concentration (abscissa) for Curve 1, corn starch at 210° F.; Curve 2, British gum at 80° F.; Curve 3, canary, British gum at 80° F.

aid of acid, about 90% soluble, and of very low viscosity; Curve 3 is a canary dextrin, of still lower viscosity, almost completely soluble, also produced by heat and acid. Curve 1 shows marked structural heterogeneity which is much more pronounced than for Curve 1, Fig. 78. This reverse S form seems to be characteristic of partially disorganized starch, as discussed elsewhere.⁵ Curve 2 likewise exhibits this peculiar form, although the existence of the small inflections admittedly is open to question. Curve 3 has the normal form for homogeneous substances and is quite similar to Curve 3 of Fig. 78. At the 10% concentration used in the case of the latter, however, it would probably be more nearly linear.

Curve 3, Fig. 80, represents the same product as in Fig. 79. The plot of the logarithm of viscosity (at 80° F.) against percentage concentration is linear

⁵ See Chapter IX.

within the concentration range shown, confirming the deductions made from the plot of the logarithm of the viscosity against $1/T$. Curve 2 (Fig. 80) represents a modified British gum from corn of character intermediate between that for Curves 1 and 2 of Fig. 79. Departure from a "square law" relationship is marked, as would be anticipated from the viscosity-temperature relationship. The rate of increase in viscosity is very great. For purposes of comparison, Curve 1 (Fig. 80) shows the logarithm of viscosity *versus* concentration of an unmodified corn starch at 210°F . in a low and narrow range of concentration. This also is linear and is indicative of relative homogeneity of liquid structure *within this narrow and very low concentration range and at temperatures near boiling* where the breaking of hydrogen bonds is greatest. Reproducible data of the kinematic viscosity for corn starch are practically impossible by any technique at concentrations of more than 5% and at temperatures much lower than the boiling point.

3. Canary Dextrins. These have already been touched upon in the discussion of British gum. They are produced by the actions of heat and $[\text{H}_3\text{O}]^+$ ions and not unreasonably may be regarded as a sort of hybrid of a highly soluble white

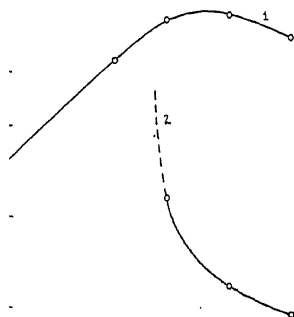
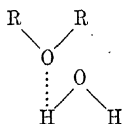


FIG. 81. Tapioca, canary dextrin conversion. Curve 1, alkali lability and Curve 2, viscosity (centistokes) plotted *versus* time of conversion in minutes (abscissa).

dextrin and British gum. This is indicated in Fig. 81 in which conversion time is plotted against the logarithm of the viscosity and against the lability in alkali. The general similarity of form of the viscosity curves of Figs. 77 and 81 is marked and is strongly suggestive of broad similarity of structural changes, especially in the early stages of conversion in which $[\text{H}_3\text{O}]^+$ ions play the leading rôle, probably

snipping off the branched chains at the 1-6 position. In the later stages (Fig. 81) of the conversion, however, the rate of change of the viscosity diminishes noticeably and the inflection of the viscosity curve (also the inflection of the curve for solubility (6)) corresponds to the peak of the curve for alkali lability, a coincidence invariably observed in the preparation of the highly soluble canary tapioca dextrins. This coincidence seems to be true also of highly soluble canary dextrins from corn starch (6). The tapioca dextrin represented by Fig. 81 is light colored. Longer conversion would result in a darker tint, a marked drop in the alkali lability curve, and only a relatively small change in the viscosity index. The solubility would remain constant in the neighborhood of 100%.

The true significance of the characteristic peak in the alkali lability curve is highly speculative. It seems to be related to both hydrolytic chain scission and to the more obscure mechanism of the structural changes caused by heat. The fact that it coincides with a sharp reduction in the rate of change in viscosity and after prolonged exposure to high temperatures seems suggestive of more profound structural modifications than occur in conversions yielding white dextrins. According to Taylor (7), "alkali-stable" substances such as inner anhydrides of the type of levoglucosan are probably being formed from chain fragments through progressive loss of hydroxyl groups, followed by consequent dissociation of chains. The increased solubility in water is promoted by formation of a multiplicity of linkages, such as:



Taylor (7) states that "torrefaction dextrins of the highly converted, yellow type, acetylate to a very small extent, indicating few, if any, hydroxyl groups."

The more perfect fluidity and homogeneity of a completely soluble, highly converted canary dextrin is strikingly illustrated in Fig. 82, in which the logarithm of the viscosity of a tapioca envelope dextrin solution of 61.5% concentration is plotted against $1/T$, between the limits 70-176° F. (294.2-353.1° K.). The rate of change of the viscosity is remarkably constant. Considering the extremely high concentration, the form of this curve lends strong support to Taylor's

hypothesis of a symbolic $\begin{array}{c} \text{R} \quad \text{R} \\ \diagdown \quad \diagup \\ \text{O} \end{array}$ structure for heat-converted dextrins.

It may seem odd that in this brief treatise, only alkali lability values, viscosity, and solubility have been mentioned as indices of dextrinization. In the experience of the author these have proved to be the most useful and suggestive tools. Neither specific rotation nor simple reducing values seem to give very useful or significant data. The rate of change of the specific rotation during conversion is not as great as for the viscosity, and the mechanism of the formation of all reducing groups and the meaning of the reducing values obtained are very

uncertain. The use of the terms "erythro- and achroodextrins" etc., which are poorly defined and meaningless, has been avoided. The subject of dextrans and dextrinization is difficult and obscure enough without adding to the confusion by the continued use of the old nomenclature. The above discussion is a superficial survey of one of the most fascinating fields of carbohydrate research.

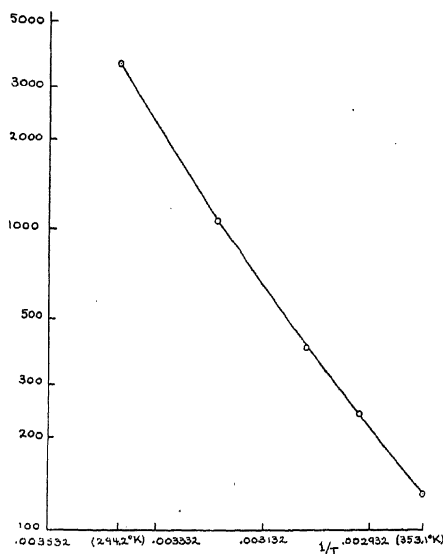


Fig. 82. Viscosity (centistokes) versus $1/T$ for a tapioca envelope dextrin at the concentration used in the application.

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CHAPTER XIII

MANUFACTURE OF DEXTRINS

1. Introduction. The name dextrin has been loosely applied to a large variety of starch degradation products intermediate in molecular weight between that average for starch and the oligosaccharides. The degradation may be by acid, heat, enzymes, or other reagents, or by a combination of any of these agencies.

Since these varied products are only remotely related chemically in the fact that they originated from starch and that they may be of the same order of magnitude in respect to molecular size, the name by itself, obviously, is meaningless unless prefixed by some modifying term which gives a clue as to the manner by which the product originated. The use of terms such as "torrefaction dextrin," and " β -amylase limit dextrin" has been proposed. This is better than no differentiation, but so cumbersome a system is nevertheless inadequate. Torrefaction dextrins, *e.g.* those made by a roasting process, show a wide variation in properties and no doubt vary greatly in their chemical composition, depending on the extent of the degradation and whether a reagent is added to alter the reaction in the roasting or heating process. Acids of various types, hydrochloric, trichloroacetic, and hypochlorous, may be added. For other uses dextrins may be made by adding alkalies, such as sodium carbonate or ammonia, and organic reagents, such as urea, in the dextrinization process. When starch alone is dextrinized by heat, it has been the custom in the starch industry to call the product a "British gum." The name gum is a misnomer but the usage is so old that it probably could not be changed without confusion and inconvenience. Considerable care therefore should be exercised to avoid confusing the various types of dextrins, because of the fact that these various starch products have been grouped under one heading.

This discussion deals with the manufacture of five classes of differently constituted starch products as outlined below. These appear to be the most important industrial types of dextrins.

2. Torrefaction Dextrins Made with No Added Catalyst (British Gums).

The simplest example of a torrefaction dextrin is the one obtained when pure starch is dried, bolted, and heated in an iron cooker with agitation. A wide variety of products results, depending on the conditions of the process. Some variable factors are (a) the type of starch used, (b) the moisture content of the starch, (c) the speed of heating up to the temperature of modification, (d) the length and manner of heating, and (e) the cooling and aging process. The chemistry and nature of some of these products have been discussed in other chapters. We are concerned here primarily with manufacture and how changes in the principal variables may be expected to alter the nature of the final product. The emphasis is primarily on corn starch dextrins.

High grade tapioca and potato starches are dextrinized to more transparent products when subsequently heated with water than those obtained from corn starch. They are less short in respect to the character of the paste and when highly converted appear to "set back" less than those of corn starch, unless the latter are additionally modified. On the other hand, commercial tapioca and some grades of potato starch appear to be dextrinized with greater difficulty than corn starch. Batches of domestic sweet potato starch dextrinized by the author were found to be converted with less ease than corn starch. This difference in starches (except as noted below) is quite likely not due to the differences in the starch *per se*, but rather to impurities associated with commercial starches.

Potato dextrins possess a characteristic odor and are not as desirable for some uses unless the starch be additionally modified.

For starches of very low moisture content, the degradation at first is essentially a physical one, and consists of a destruction of the orientation of the molecules in the original granule along definite lines of cleavage. Some scission in the molecular chains then follows probably at specific points that are under the greatest strain. Rearrangements follow next, quite possibly to form additional branched structures through intermolecular condensation and glucosan types of structure by intramolecular condensation.¹ The result is a more freely dispersing starch product with high adhesive strength for a given order of molecular magnitude. If the moisture of the starch is not reduced to very low levels and if the final temperature of dextrinization is rapidly approached in a poorly ventilated cooker, new molecular orientations result which are possibly akin to those that occur in a limited gelatinization of starch. Furthermore, for corn and similar starches at least, the acid activity of the "combined" fatty acids becomes sufficient to induce some additional hydrolytic cleavage. Hydrolytic cleavage could not be expected to be completely eliminated in this type of conversion, in any case, since the activity of the water present at these temperatures would by itself be expected to induce some scission. The result with some starches is that the dextrin does not disperse as readily in water and, at a given hot paste viscosity, tends to "set back" more on cooling. The extreme case of this type is obtained by heating starch in a pressure cooker at a high relative humidity with superheated steam. Very curious products result by this process. For example, even potato starch may be converted into a product which disperses less freely in water than native starch, and when dispersed it sets to a rigid gel according to reports of Sair.² If ventilation in the cooker is ample during the heating up period and the latter is not too rapid, then obviously the end-products will more closely approach those made in a faster dextrinization process with highly dried starch. Instead of ventilation, a partial vacuum may be applied to accomplish the same results. Most of the benefits claimed for dextrinization³ under a vacuum are no doubt due to a speedy and continuous removal of moist^{ing} as claimed by Krause (3).

Depending on the length of the heating period and the temperature employ^{rin} variable products result. Some at one extreme differ little in physical charact^{istics} from the starches (thin boiling). At the other extreme are those whic^s have a very dark color and the odor of caramelized sugar. The solubility of the dextrin in cold water increases with the time of conversion. Indeed, all of these commercial dextrins, during the intermediate stages of the above conversion,

¹ The work of Frahm (1) would suggest that condensation through 1-6 glucosidic bonds results first and that glucosans form only in the most extensive stage of the heat treatment. Brimhall (2) proposes rearrangements through 1-6 glucosidic linkages to form large branched structures.

² Project reports to the Corn Industries Research Foundation, the data of which have not been published.

are chemically very heterogeneous. Intermediate samples contain portions of cold water-soluble constituents evidently of quite low molecular weight. There are also found starch granules which have apparently been altered very little in the heating process, for all granules do not possess the same resistance to destructive agents. Small granules in a starch sample are noticeably more resistant than the largest granules. It is perhaps because commercial dextrins present such a heterogeneous composition that the subject of dextrinization by heat has attracted so little attention by those interested in fundamental research.

Dextrinization is an art. Each manufacturing concern seems to have developed its own preferred procedures for making products to supply the trade. These processes have been developed by and the products defined according to empirical methods and procedures. The course of the dextrinization is usually followed by (a) the reduction in the viscosity of the product when a paste is formed in water, (b) the increase in the water-soluble content, (c) the changes in color, and (d) the change in the amount of reducing substances in the water-soluble extract. However, as might be anticipated, the product of one manufacturer is frequently not identical to that of another in its working characteristics or colloidal behavior although they correspond in respect to the values determined by the tests mentioned above. Also, one product may match another according to one or more of the above tests but may differ in regard to others.

However, in general, the process of dextrinization may be summed up as follows: After the starch is prepared, as described in Chapters II to V, it is ground in a suitable mill and passed over fine bolting silk. It then passes to equipment for redrying. A Huhn type drier is suitable for this operation. In this equipment the starch is reduced to 5% moisture content or less. It is then passed to large iron kettles provided with efficient agitation. The kettle may be loosely covered or, when vacuum or pressure is to be applied, tightly covered. Indirect heating of the kettle is preferred, owing to the fire hazard involved in heating a very dry, highly combustible substance such as starch. For this purpose the kettle may be jacketed for the circulation of steam at high pressure or of hot oil. Gas-fired kettles are still used to some extent, however. In any event, the heating should be as uniformly applied as possible and care should be taken in the design of the kettle to reduce local overheating to a minimum. For starch of low moisture content, the temperature may be raised to about 120–130° C. as fast as is consistent with the design of the cooker in respect to heating and agitation. At this temperature the heating rate should be reduced to allow the removal of the associated water, which is released at this temperature, as uniformly as possible and to avoid undue local overheating which becomes more difficult to avoid as the temperature is raised into the range necessary to produce these types of dextrins. Frequently, temperatures of dextrinization in the range of 175° C. are used, and some manufacturers prefer a shorter conversion time at temperatures in the neighborhood of 200° C. Gas-fired kettles are generally used for these short time conversions at high temperature.

The modification is allowed to proceed until the proper characteristics develop; *i.e.*, viscosity, percentage of soluble material, and color. The conversion is then stopped by cooling the dextrin. For this purpose it is usually dropped quickly to a cold water-jacketed drum in which agitation is provided to facilitate the cooling. The product is naturally very dry and hygroscopic. Some manufacturers humidify the starch at this point with moist air. Others prefer to allow the product to return to equilibrium with the atmospheric moisture naturally by an aging or storage process. The latter process has an advantage for the manufacture of certain types of dextrans in that, simultaneously with water absorption, the dextrin releases a good portion of gases that were adsorbed during dextrinization. These gases are to a large extent composed of carbon dioxide, which, if present in a "green" or unaged dextrin, will be released when the dextrin is cooked with water and then tend to create an undesirable amount of foam.

The dextrin may be blended with chemicals or further treated with reagents to produce specific changes in the colloidal characteristics or working properties of the dextrin. For this purpose a large blender or mixer is used which, if of sufficient size, serves to homogenize the product from several dextrinizing kettles or cookers and thus insures uniformity in the product (according to the specifications mentioned). For example, borax may be blended with the dextrin to modify its flow characteristics when it is cooked into a size or adhesive or to increase its speed of drying. It has been noted in fundamental studies that the addition of borax to solutions of amylose tends to prevent gelation and spontaneous precipitation. Among other paste-modifying agents used the following may be mentioned: Henkel and Cie (4) add water-soluble phosphates; Bloede adds bisulfite (5); Wakeman (6) suggests the use of borax and a liquid plasticizer such as diethylene glycol; Bauer *et al.* (7) suggest the use of borax and a liquid plasticizer to give the gum better working properties.

Equipment suitable for the humidification and homogenizing operations have been described by several workers (8-10).

Before being finally packaged, the dextrin is bolted over fine silk, with grinding if necessary, in order to remove specks such as metallic scale from the dextrin kettle, particles of overheated starch, etc.

In all of the operations described above a very dry and dusty product is under considerable agitation. Elaborate dust-collecting systems, therefore, are used to gather and recover the starch. The atmosphere containing the starch dust is often scrubbed with water. The solids are frequently filtered out, dried, and returned to an appropriate point in the process. Momentary neglect of the operation of dust-collecting systems or of means to prevent the escape of starch dust has frequently had disastrous consequences in the destruction of life and property. Dry starch dust is a continual fire and explosion hazard. Good housekeeping and an efficient means of collecting dust is essential in any dextrin-manufacturing plant.

Variations from those outlined in the methods or equipment used in the dextrin process will be found in the patent literature. Very early, Wilson and O'Reilly (11) suggested a continuous method or process for dextrinization. Verity (12) heated starch in shallow closed containers immersed in a heated liquid in order to secure more uniform heating. Henkel and Cie (13) described a method for dextrinizing starch by pressing it between hot surfaces. Horesi (14) has patented a process wherein the dried starch is dextrinized by suspension in a very hot atmosphere. The process is a continuous one. Bode (15) described a method by which starch is completely dehydrated, suspended in a water-immiscible solvent, and mixed with hot oil in which it is dextrinized.

3. Torrefaction Dextrins Made with Acid Catalysts. Frequently, a small amount of acid may be added to the starch to speed up the dextrinization. Such products may be referred to as modified British gums. On the other hand, substantial amounts of acid may be added to the starch, and the products so formed are called the white dextrins.

These products vary from the gums described in the preceding section, depending on the amount of active acid used in the process. Degradation by hydrolytic scission is undoubtedly more pronounced in this type of dextrin. To arrive at products with a given viscosity and solubility for the same heating time a lower temperature of conversion is required than when no acid is used. As a result, these acid-made dextrins are considerably lighter in color than the corresponding gums. Products of extremely low viscosity which are usable when cooked with water in a ratio of 1 : 1 (or even less) finally result. These products are the remoistening gums of commerce; *i.e.*, adhesives used on labels, envelopes, postage stamps, tape, and similar articles. Some of the end-products of this type of conversion are referred to in the trade as canary dextrins.

In the acid process the content of reducing sugars in the water-soluble fraction slowly increases to a maximum and then falls off in the last stages of the conversion. It is evident, therefore, that in this process a third reaction is in progress in addition to those discussed above under gums. As the sugar content increases under the influence of the acid, the latter also catalyzes a condensation or polymerization of sugars into polysaccharides which probably do not exist in the parent starch. Whether these polymerization products are of the gentiobiose type such as result when dextrose solutions are polymerized with acid³ or whether they are of a more complex nature such as those obtained when dextrose is polymerized in the dry state with other catalysts⁴ has not been ascertained. However, the extent to which this polymerization is induced depends on the activity of the acid added and on the extent of the conversion.

Hydrochloric acid is the preferred reagent for this type of dextrinization, because its acid activity is high and hence requires that little be added, because

³ The polymerization of dextrose by acids is discussed in sections treating with acid hydrolysis of starch. For further reference see the article by Berlin (16).

⁴ It may be, however, that polymerization in the dry state also involves the formation of 1-6 glucosidic bonds. (See also Frahm (1).)

the acid is volatilized to some extent as the dextrinization proceeds and therefore neutralization of the end-product may in some cases be omitted, and because light colored products result.

Other acids may be used, however, such as lactic (17), phosphoric, and sulfuric, but more care in application is required. Otherwise, these acids tend to cause local overtreatment and to produce an increased amount of dark colored specks in the final product. The author has experimented to some extent with conversions with phosphoric acid and has found that satisfactory products which cooked to pastes of low viscosity and high mobility can be produced from corn starch by incorporating the acid with the starch in water suspension, and then filtering and drying the starch. Spraying the acid on dry starch gave poor results in the type of dextrinization equipment used. Another acid which appears to have some merits with corn starch, compared to hydrochloric acid, is trichloroacetic acid. Bulfer and Gapen (18) suggest the use of monochloroacetic acid and chlorine. Acetic acid vapors were recommended in an early patent by O'Neill (19).

Salts such as CaCl_2 may be used (20). The CaCl_2 not only appears to catalyze the reaction of corn starch but its presence in the final product alters the colloidal properties of the cooked dextrin. If the correct amount of the salt is present, the dextrin appears to be more highly dispersed and more mobile at a given viscosity (21).

Acids or acid vapors which possess an oxidizing function may be used. Nitric acid (19), hypochlorous acid, and chlorine gas are examples of this type of reagent and, as might be anticipated, they alter the colloidal properties of the cooked dextrin. The color of dextrins treated with nitric acid is not as excellent as that produced by hydrochloric acid. The author has experimented at length with hypochlorous acid-converted dextrins in order to find a substitute method for producing the relatively expensive oxidized corn starches of commerce. The starch, in water suspension, is saturated with gaseous chlorine as described in a patent recently issued to Kerr (22). After several hours contact with the acid at room temperature, the slurry is filtered without neutralizing, and the product is dried, bolted, and dextrinized without further additions. Superior products result as compared to those of conversions with hydrochloric acid. However, it appears that a substantial proportion of the degradation is hydrolytic.

It is no small problem to promote the oxidation reaction during the dextrinization of starch. The colloidal properties of oxidized corn starches are excellent. If a similar product could be produced by dextrinization, costs might be reduced; and if the properties of oxidized corn starches could be carried into the range of the dextrins of high fluidity, these dextrins would find a ready market. It would appear that the patent of Berquist (23) might accomplish the first aim. By this method highly dried starch is treated with chlorine gas in the dextrinization procedures. The reason this method has not been more extensively used may be the difficulties arising in handling chlorine gas in the usual type of dextrinizing equipment. Fuller (24-26) partly oxidizes the starch with calcium

hypochlorite and then dextrinizes it in the presence of various amounts of acid. It is claimed that the presence of CaCl_2 formed in the reaction also catalyzes the dextrinization. It is obvious, of course, that more or less hydrolytic scission is involved in this process.

It would seem that the second object mentioned above might be accomplished by oxidizing the starch, drying, and completing the degradation by heat alone. The very surprising result is, however, that these oxidized starches are much less susceptible to dextrinization by heat than untreated starch. It is very difficult to reduce the viscosity of an extensively oxidized corn starch to a lower value except by the use of conditions which cause a general breakdown in the starch with the production of highly colored products. Reducing the extent of the preoxidation results in a deviation from the properties of oxidized starches in proportion to the reduction in the amount of the preoxidation.

The method of manufacture of the white dextrins varies little from the methods given in the first section except for the addition of two steps (the second of which is sometimes omitted): addition of the acid catalyst and neutralization.

The usual method of incorporating an acid, such as hydrochloric, is to atomize it into a large closed wooden mixer provided with efficient agitation for the starch. An aqueous solution of 10 to 15% HCl is commonly used for atomizing. After the acid treatment the starch may be dried. It is the preferred process, however, to use very dry starch and to use acid of as high strength as is consistent with the design of the mixer for incorporating the acid uniformly with the starch. A short period of storage completes the intimate dispersion of the HCl into the starch sample.

Acid and acid catalysts may be thoroughly mixed with the starch by adding the acid to the starch in water suspension. This is followed by filtering, drying, and grinding (27-30). Perfect mixing of the starch and acid is accomplished by such a procedure, which seems to be required for acids such as phosphoric acid, when used at high concentrations. However, excessive quantities of acid must be used, since some is removed in the filtration operation and in the case of volatile acids some is lost in the drying operation.

If extensive storage of the acidified starch is employed, hydrolytic degradations will be increased in proportion to the storage time. Indeed, starches such as corn and in particular potato, will lose viscosity progressively with storage at room temperature at a pH as high as 4.0.

As mentioned before, the temperature, the time of dextrinization, or both, may be reduced in dextrinization with acid. Again, as in the case of gums, different combinations of time and temperature result in products with different properties. The possible variation in the acid concentration is another variable which further multiplies the number of possible products. Hence, each manufacturer of dextrin has available a long list of products produced by various combinations of variables which products can be used to supply almost any specific requirement.

The dextrins made at higher acid concentrations are frequently neutralized after conversion but those made with lesser amounts of acid are not so frequently neutralized. The result is that some dextrins of rather low pH appear on the market. This fact should be borne in mind by the user when the pH of his product or application is an important factor. He should either adjust the pH of the dextrin, during or after cooking, to the range which is consistent with providing best results in his particular application, or else he should inform the manufacturer of the pH desired in the product.

Elimination of the neutralization step has one disadvantage and one advantage in addition to the obvious advantage of a reduction in the number of steps in the manufacturing process. The disadvantage is that stability of the product, particularly the thicker dextrins, is less during a prolonged storage period before use. The advantage is that, if the product is left acid, the user completes the modification or degradation of the starch when he cooks the product. Shorter, or less drastic dextrinization procedures are therefore required, thus speeding production. If the user desires to adjust the pH of the product to suit his own wishes, this should be done after cooking. If neutralization is brought about before cooking, a slightly thicker product may result than he is able to use.

In the simplest case, neutralization may be accomplished by a dry blending with alkaline reagents. Sodium carbonate may be used. This, however, is not preferred procedure, since the dextrin is not actually neutralized. The result is rather that its average pH is neutral. Furthermore, the tendency to produce a foamy product obviously is increased. Phosphate may also be used. Also, alkaline modifying agents such as borax are to be blended with the product, slight addition will of course neutralize the original acidity.

Dextrins may be more perfectly neutralized by exposure to alkaline vapors such as ammonia. This action may be accomplished by placing the dextrin in shallow containers in a large chamber into which the ammonia is introduced, or it may be accomplished in mixers provided with agitation into which ammonia gas is blown or aqueous ammonia is atomized. If the dextrin is to be artificially humidified, it can be accomplished during this latter type of neutralization.

Many variations of the basic process outlined above are concerned with other methods of introducing the acid catalyst. Browning and Barlow (31) and also Fielding (32) introduced acid vapors into the starch after it was placed in the dextrin kettle. In a subsequent patent Fielding (33) recommended a very dry starch for this purpose. Lenders (34) suggested adding the acid catalyst progressively to the starch as it was introduced into the dextrin kettle. Waulkan (35) would acidify a part of the starch with the required acid and add this mixture to the balance of the starch. Edson (36) found that better dextrinization could be accomplished by blending the starch and acid and heating the mixture in thin layers. Fuller (37) has described a novel process which consists in gelatinizing the starch with alkali, drying and grinding, then treating alkaline starch with chlorine and heat. Stein, Hall Mfg. Co. (38) secured beneficial results by adding a gel-inhibiting substance, such as SO_2 , to starch of normal moisture

content. The catalyst is then added and the mixture dextrinized. Meyer (39) has patented a process in which a volatile organic acid is used for dextrinizing and recovered by distillation and condensation. Stutzke (40) treated starch with nascent chlorine ($\text{HCl} + \text{HNO}_3$) for several hours, and then forced the starch through coils at about 1000 lbs. pressure. A double dextrinization is described in patents issued to Bruce (41, 42). In order to improve the colloidal properties of remoistening gums and other dextrans, the latter, after initial steps as outlined in the previous sections, are cooked under pressure with water until the desired properties are produced. In order to reduce the sugars present in dextrans, Mathiessen and Krieger (43) suggested that a fermentation step be appended. More recently hydrogenation at high pressures and temperatures has been suggested to eliminate reducible substances (44). To produce dextrans free from lumping tendencies and other undesirable characteristics, Coppock (45) recommended additionally treating the dextrin by subjecting it to a pressure of about 1 lb. per square inch between heated plates at 70–130° F. for 3 to 15 min., then grinding and bolting.

4. Torrefaction Dextrans Made with Basic Catalysts. Basic catalysts are employed to produce gums with a minimum of hydrolytic degradation during the process. In the simplest case, a small amount of sodium carbonate is blended with the starch in the wet state before it is dried or with the dry starch. Other alkaline buffers may be used which neutralize both the acids associated with, or liberated from, the starch and the acids produced by heat degradation of the carbohydrate. It is quite possible that an appreciable atmospheric oxidation is also induced by heating the starch under alkaline conditions. In this manner dextrans are produced with rather high molecular weights and paste body for a given content of water-soluble material. Or stated differently, highly dispersible gums are produced which still possess high viscosity or high "water-carrying" power. These gums are used particularly in sizing.

The usual types are produced by methods quite analogous to those already outlined in the preceding sections. If the alkaline buffer is carefully incorporated by dissolving it in a starch slurry and if the starch is carefully dried to a low moisture content before the application of dextrinizing temperatures, the dextrinization may be carried into the range of highly soluble products without the production of excessively colored substances that might be expected from an alkaline roasting procedure.

An improvement in the general process and in the quality of gums produced is claimed in a patent issued to Clegg and Hilliard (46). This patent describes the use of basically reacting ammonia compounds.

A substantial improvement in technique and product is described by Caesar (47). An outline of a typical example, to illustrate the process, is given. 18 lbs. of starch are heated to 132° C. in the dextrin kettle. At this temperature 408 g. of urea are mixed in and the temperature is raised to 193° C. over a total period of 4 hrs. The urea melts at about the temperature at which it is stirred into the starch, so that the granules become coated with the melted urea. Rising temper-

atures then serve to decompose the urea into ammonia and products which eventually liberate more ammonia. The latter is, therefore, generated directly on the granules as they are being dextrinized and thus hydrolytic degradations, with the attendant loss in dextrin paste body, are eliminated. It is claimed that dextrans are produced which readily disperse in cold water to give liquids of high stability to "set back" and relatively high paste body.

Another variant of the general process described is disclosed by Haake and Haake (48). In this method alkaline hypochlorites are blended into the dry starch with mixing and the mixture dextrinized by heating. Products resembling the oxidized starches are said to result from such a procedure.

5. Wet Dextrinization with Acid. Wet dextrinization with acid is accomplished by two general processes, (a) one in which the starch is gelatinized and a dextrin solution results and (b) another in which the starch granule is not gelatinized during the process. In both processes, obviously, hydrolytic degradation predominates. The former method is discussed under acid hydrolysis and the product is referred to in the United States as a corn sirup, unmixed (C.S.U.), when corn starch is used, rather than a dextrin. When the product is to be used as a liquid dextrin, the conversion with acid is usually stopped at an earlier period in the hydrolysis than when the product is to be used essentially or its sugar content. Hence, these dextrans are referred to as unmixed corn sirup of low purity (low percentage of reducing sugars calculated as dextrose).

A variation in the method produces a dehydrated product. After the initial conversion with acid in a water medium (usually with steam pressure in a closed converter to conserve time, or acid, or both) the fluid is neutralized and passed between heated rolls, spray-dried, or flash-dried. The dehydrated product is then ground and bolted. Such a product, containing about 5% of reducing substances estimated as dextrose, is sold by one firm under the trade name of Amidex.

The second wet dextrinization method mentioned is discussed under modified starches. It is an extension of the process used for making the thin boiling starches.

The starch is stirred with water to form a slurry, and a converting acid is incorporated. Hydrochloric or nitric acid is suitable. The starch may then be partially converted by raising the temperature to between 50° and 55° C. for several hours. The converted product is filtered, without neutralization, and the wet cake is placed in converting kilns where it is further dehydrated and converted over a period of several days. The secondary conversion should not be speeded up by applying high temperatures at the start, or the starch will partially gelatinize and a gritty product will result.

The preliminary conversion should not be extended to the point at which the amount of cold water-soluble products is appreciable, since, if it is, these products are lost in the filtration operation and pass into the filtrate. In a modification of the process the initial conversion is practically eliminated and all of the conversion is performed in the kilns.

The final conversion is extended until the desired characteristics result. These vary depending on the application. The end-products are characterized by their content of water-soluble material and by their paste viscosity. Products giving a 100 g. Scott viscosity test of 50 to 75 are made with the soluble fraction as low as 10%. Others contain 30 to 40% of soluble material and give a 100 g. Scott test of 30 to 35.

The final product may be neutralized by exposure to ammonia fumes, as described in previous sections, or it may not be neutralized, depending on the use intended.

This method of conversion has been practiced for many years in the United States with but minor variations from the process originally described by Lenders (49). The product has had an ever increasing market and to-day is sold in large volume for sizing purposes, particularly for finishing textiles and for sizing paper products. Although, some grades are as much as 30 to 40% water-soluble and have a reducing value as high as 19 (by alkaline ferricyanide reduction),⁵ which may be compared to a value of 1.6 for starch and 269 for maltose, the product is referred to in industrial practice as a special starch. One such product is sold under the trade name Foxhead starch.

A variation in the above method has been described by Stutzke (30). The starch, suspended in aqueous acid, is simultaneously converted and dehydrated by spraying the suspension into superheated steam at about 250° F.

6. Dextrinization by Enzymes. These conversions are described in further detail under "Modification of starch by enzymes" (Chapter XVI). The modification is more often performed by the user at the time of application. The notable exception is the adhesive manufacturer, who prepares liquid or semi-dehydrated pastes and glues, either by simply converting the starch enzymically or by mixing this product with other dextrans for particular uses. The literature contains many references to the use of various types of enzymes for the production of products referred to as "dextrans," but in some cases the products formed only vaguely resemble the carbohydrate chemists' conception of a dextrin, if indeed the concept is possible to define at all. One of these types, the so called Schardinger dextrans, has been discussed in the chapter dealing with the chemistry of starch (Chapter VIII).

The dextrans produced by enzyme action which are of present industrial importance are made by the action of the two enzyme types, α - and β -amylase, either separately or by their combined activity in such natural mixtures as malt extracts. β -Amylase, in addition to producing a small yield of a dextrin of high molecular weight from starch, known as β -amylase limit dextrin, simultaneously produces large amounts of sugars of low molecular weight such as maltose. α -Amylase is apparently able to break into the starch molecules and set free products with longer chains than those of the sugars. Some of these may exhibit a reducing value but they are not, in the general sense, sugars. The amount of actual sugar produced is very small. Therefore, α -amylase has been

⁵ Cubic centimeters of 0.1 N ferricyanide per gram of carbohydrate.

termed the dextrinogenic amylase, while β -amylase is the saccharogenic amylase.

In industrial practice it is not practical to separate the two components, or types, from the mixtures in which they naturally occur. However, some sources may be decidedly richer in one type of component than another. Therefore, naturally occurring mixtures are commonly used, and conditions are employed which favor the action of the dextrinogenic or retard the activity of the saccharogenic component. β -Amylase appears to be the less sensitive to destruction by acids but is more easily inactivated by heat.

Neither enzyme appears to act rapidly on ungelatinized starch. The starch may be gelatinized by heat, which process if carried out in the presence of the added enzyme tends partly to inactivate the β constituent. The gelatinization temperature of starch is, moreover, a function of pH, and within limits, the higher the pH, the lower is the gelatinization temperature. In practice, the industrial conversion usually is carried out by adding the enzyme to a slurry of raw starch and water and heating the mixture. Then, as the starch starts to gelatinize, it is converted. It is less economical to gelatinize the starch by heat and cool to a temperature at which the enzyme activity is greatest and the rate of destruction is least, even were it otherwise desirable to do so. Conditions employed, therefore, are a balance between those required to gelatinize the starch on the one hand and those required for stability and activity of the enzyme on the other, for both α and β components are inactivated by heat even though the β component is the more sensitive.

An example is given further to illustrate this discussion. For certain sizing operations corn starch is made up as a 20% slurry with water. The pH is then adjusted to that for the optimal dextrinogenic activity of the enzyme preparation employed. For most commercial preparations this is between pH 6.0 and 6.5. Between 0.2 and 0.5% of enzyme is added, based on the dry weight of starch. The amount added will depend on the potency of the enzyme preparation and the extent of the modification desired. In some cases quantities of enzyme as high as 1% or even higher may be required. The mixture is then stirred in a wooden or iron tub which should be free from fittings of enzyme-inhibiting metals, such as copper and brass. Heating is started. This may be done by the use of open steam jets. The temperature is raised to about 70° C. in about 15 min. and then is maintained constant for 15 to 30 min. to complete the desired conversion. The remaining active enzyme is destroyed, and the dispersion of the dextrinized starch is completed by bringing the temperature to about the boiling point. The conversion is adjusted to the proper dry solids content usually by dilution but in rare cases by evaporation.

The writer has prepared many of these enzyme-converted dextrins in a dehydrated form by roll-drying and by spray-drying the conversion liquors. A small amount of borax may be included in the conversion liquors before the drying to facilitate the wetting properties of the dried dextrin, to modify its body, and to increase its drying qualities when it is to be used as an adhesive after redispersion in water.

Variations in the above process will be desirable, depending on the starch used, the sensitivity of the enzyme preparation to heat, and the pH required for peak efficiency of the enzyme. Information concerning the latter two variables is usually supplied by the enzyme manufacturer. Lower temperatures, or a lower pH, or a combination of lower temperatures and a lower pH value may be used, particularly when a non-cereal starch is used such as tapioca, which gelatinizes more readily than corn starch. However, in so doing, one runs the risk of allowing greater activity of the saccharogenic factor, and the unrestricted production of sugars detracts from the strength of the dextrin produced. β -Amylase has a peak activity between 45–50° C., at pH between 4.5 and 5.0.

Adhesive manufacturers have studied this process in detail and have developed variations to produce special effects or to obtain economies in operation. Greater enzyme activity may be obtained and possibly higher starch to water ratios may be used, if the enzyme is added progressively in small portions as the heating proceeds and if the increase in the temperature takes place slowly. High starch concentrations are desirable, since certain manufacturers partially dehydrate the final product by evaporation. The more water present, the more costly is the process.

An extreme case was studied by the writer. An attempt was made to convert a starch slurry made of 1 part of starch to 2 parts of water. In order to avoid the production of an unmanageable plastic mass in the early stages of conversion, due to a gelatinization of unconverted starch, it became necessary to extend the preliminary heating period over several hours time. The enzyme was added progressively in five equal portions. One addition an hour was made at 100°, 125°, 135°, 145°, and finally at about 155° F., when it appeared that the conversion was in the last stages. The surprising result was obtained that the dextrin had very little strength and that sugar production was excessive. It was subsequently found and reported (50) that in a delayed treatment of starch at temperatures below the commonly accepted gelatinization point the soluble end-products are almost exclusively highly fermentable sugars.

Dextrins which tend to revert less and remain free flowing are produced from starch according to an elaborate modification described by Singer (51). Starch is mixed with water at a concentration of 25% (1 part of starch to 3 parts of water) and heated to 149° F. as rapidly as possible. A quantity of malt extract (4.5% based on the weight of starch) is added. The malt extract has a diastatic strength of only 75° Lintner. The temperature is rapidly raised to 158° F. and then raised about 2° F. per minute until 176° F. is reached. The mass is then brought to boiling quickly. The conversion mixture is heated under pressure at 280° F. for 5 min. It is cooled to 176° F., and 18% more malt extract is added. The temperature is held at 176° F. for 25 min., whereupon the solution is again brought to boiling temperature. A very thin dextrin results, suitable for such uses as a remoistening gum.

As with the other types of dextrins described, various plasticizing agents, retrogradation retardants, and colloid modifiers may be added to or incorporated

in the final product of enzymic dextrinization. Representatives of these modifying agents are urea, dimethylol urea (52, 53), dicyandiamide (54), hexalin, borax, and calcium chloride.

From the discussion it should be apparent that the type of conversion process used to produce dextrins by enzymes differs in important details from the diastatic conversion of starch employed by the fermentation industries. The two processes should not be confused. Attempts to employ practices found in the literature for the latter, such as the use of various enzyme accelerators or stabilizers or the use of a more acid conversion medium may lead to disappointing results.

Special equipment or improvement in the design of equipment used in the various steps of dextrin manufacture, in addition to those already discussed, is described in several patents (3, 55-60).

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CHAPTER XIV

ACID HYDROLYSIS OF STARCH

1. Introduction. The discovery that starch will yield sugar-like substances when treated with acid is apparently attributed to the Russian chemist, Kirchoff (1). His experiments, reported in 1811, established the basis for the commercial production of starch sirups and the crude starch sugars. That the reaction is a hydrolysis and that the end-product from starch is identical to grape sugar or glucose were determined a short time later by de Saussure (2). The refined sugar is referred to as dextrose in American practice.

Attempts to study the mechanism of the reaction have been complicated mainly by three factors: failure to recognize and appreciate the importance of secondary reactions; lack of suitable analytical methods to study the system of reactions; and a lack of knowledge concerning the chemical nature of the substrate, starch. The result is that most of the early work, in common with many other phases of starch chemistry, is of little value and is of interest mainly to the historian.

2. Starch As the Substrate in Hydrolysis. The early attempts to determine the chemical composition of the starches and the constitution of starch components have been discussed in a previous chapter. The confusion which has

prevailed during the past century in respect to this subject has been indicated. Very considerable progress has been made within the last decade, however, in that the principal problems involved have apparently been solved. It has been established that the starches, in general, are composed of two molecular species, although the proportion of each no doubt varies from starch to starch, depending on its biological origin. The component species occurring in smaller amounts (and lacking in several starches) consists of chains of 1-4 α -glucosidically linked glucopyranose units. These species are found to vary in length from possibly 100 to 400 or 500 glucopyranose units. The balance of starch has a more complex structure which is probably of much greater molecular weight. In addition to the maltose type of linkage, evidence points to branching or ramification through 1-6 α -glucosidic linkages. Estimates of the molecular magnitude of such giant molecules and a determination of their exact structure are extremely difficult. It would seem likely that the order of magnitude for molecular weight averages about 10 times that of the smaller component. That the pattern of the more complex fraction varies from starch to starch would seem to be settled. Whether this pattern varies within a starch, that is, whether varying degrees of branching exist so that any one starch may be considered to be composed of a series of molecular species with increasingly complex structures, or whether the variation in behavior noted for the two major species of a given starch is of molecular magnitude only, is an unsettled question. For the sake of discussion, however, that part of starch which is of lower molecular magnitude and which behaves as though it were a chain structure will be referred to as the "linear fraction." The balance will be referred to as the "branched fraction." The former also may be called amylose and the latter amylopectin, although it should be borne in mind that this usage does not necessarily correspond to the varied use which has been made of these terms to denote starch fractions during the last 40 yrs.

It has been established that all starches are not entirely composed of carbohydrate. Some starches, such as potato, contain molecules of amylopectin which are esterified with phosphoric acid. Other starches, such as corn, contain non-carbohydrate residues, *e.g.* fatty acids, so closely associated with the carbohydrate that they cannot be removed from the latter in the usual process of starch manufacture or in the laboratory with the common solvents for such substances. Corn starch, with the hydrolysis of which this chapter is chiefly concerned, is composed of about 30% amylose. At least a third of this amylose is undisputably linear in structure. The amylopectin is probably phosphate-free but the whole starch has associated with it, no doubt by adsorption, inorganic phosphates, the cation of which varies according to the starch manufacturing process. Fatty acids and nitrogenous bodies of a protein nature are also present.

Theoretically, the hydrolysis of any one starch is a composite of at least two reactions: the hydrolysis of linear chains of comparatively low molecular weight, and the hydrolysis of very large, branched structures. A fundamental study of the hydrolytic reaction should logically proceed from a study of each reaction separately to a study of the hydrolysis of a mixture of these two molecular species.

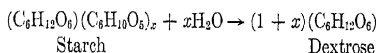
Practically no information of this nature has been reported, since these fractions have not been available in even relatively pure form until quite recently. Most of the more recent work has proceeded on the assumption that the primary reaction of hydrolysis may be viewed as an acid-catalyzed hydrolysis of 1-4 α -glucosidic linkages and that each of these bonds in starch is equivalent to the next. Little thought has been given to the availability of these various bonds or to the effect of other physical variables which might tend to impede or accelerate their scission, such as differences which arise from the fact that these bonds are links in either a linear or branched structure. In respect to availability, it would seem probable that nearly 10% of corn starch exists in a state so completely oriented that it is solubilized with difficulty in the presence of acid at the temperatures used in commercial starch hydrolyses. This portion is predominantly linear in configuration. It may be recovered as an insoluble precipitate, along with other insoluble residues, in the early stages of the hydrolysis by stopping the reaction, cooling, and filtering. Practically no information exists as to the relative rate of hydrolysis of this fraction by acid although its hydrolysis by enzymes has been the subject of several reports. Evidence pointing to the fact that some of the glucosidic linkages in starch are more susceptible to hydrolysis than others has been presented in previous chapters. This observation may account for the more rapid hydrolysis of starch in the initial part of the reaction, and the conclusion conforms with the results reported by Haworth (3) that the "disaggregation" (initial hydrolysis) of starch involves a slightly lower activation energy than that for the normal, 1-4 α -glucosidic bond.

But it is assumed, furthermore, that the small number of anomalous linkages are of minor importance in a study of the kinetics of starch hydrolysis. Some workers (4) have assumed that the 1-6 α -glucosidic bond hydrolyzes at a rate at least equivalent to that of the normal 1-4 α variety. Others (5, 6) have sought to show that the former linkages are more resistant to acid than the normal linkages, but pertinent experimental data on the subject are lacking.

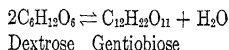
3. Reactions Involved When Starch Is Treated with Acid. An examination of the end-products of the reaction discloses the presence of glucose as the major product. In addition, depending on the conditions imposed, there are present variable quantities of gentiobiose, the isomer of gentiobiose containing a 1-6 α -glucosidic linkage, derivatives of furfural, levulinic acid, and other degradation products which result from treating carbohydrates with acid at high temperatures. In addition to the main reaction which results in the production of homologues of maltose, maltose, and finally glucose, there are formed also by hydrolysis a series of higher sugars containing at least one 1-6 α -glucosidic bond in their structure which give rise to the isogentiobiose mentioned. In addition to the primary reaction of hydrolysis, at least two other types of reactions are in progress. First, it is evident from the work of Berlin (7) and others that the gentiobiose, which can be isolated from starch conversion liquors, can be accounted for by a rejoining of 2 glucose molecules into a disaccharide containing a 1-6 β -glucosidic bond. Secondly, it would appear that after glucose is formed it may be degraded

in the presence of acid catalysts at high temperatures to non-carbohydrate bodies. Obviously, both of these secondary reactions detract not only from the yield of dextrose, maltose, and related sugars, but also from the quality of such products in respect to taste and color, particularly when the end-product is finished as a sirup of high sugar content. When the sugar is crystallized from the conversion liquors, the mother liquor, or "hydrol" as it is referred to in dextrose manufacture, is of unpleasant taste, and its utility is restricted because of the undesirable properties of the by-products present. In consequence there is a very material decrease in the yield of sugar.

To facilitate discussion, the primary reaction may be represented by the equation



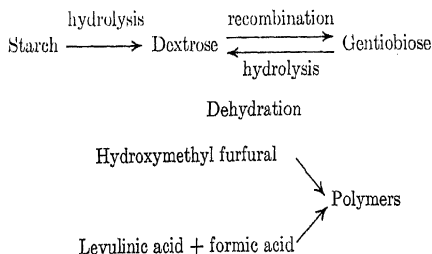
As the concentration of dextrose increases, the first secondary reaction is induced to proceed to the right:



It is also quite possible that dextrose may rejoin with maltose and its homologues by means of 1-6 β -glucosidic linkages as well. This reaction is reversible (8). The reconversion of "hydrol" to increase the yield of dextrose has long been known to the industry (9). The primary reaction is obviously not reversible, since dextrose reacts in the presence of acid to form the gentiobiose type of polymer.

The secondary reaction involving the production of the furfural type of dehydration by sugars in the presence of acids, in the case of dextrose, most likely results in the formation of hydroxymethyl furfural. It is also quite possible that the yields of levulinic acid in the acid treatment of starch and dextrose, discussed recently by Moyer (10), may originate by way of hydroxymethyl furfural. The last two products may polymerize to substances of unknown composition. In addition to these products, others of low molecular weight may also result.

Diagrammatically, then, the action of acid on starch may be represented in its essentials by the accompanying composite scheme.



Because of the complexity indicated, it would seem extremely difficult to study the process quantitatively. Within the range of conditions employed in practice, however, the vertically written reaction occurs to only a fraction of 1%. Hence it is possible, by confining the study to the limitations of commercial practice, to obtain approximate results for the two major reactions, hydrolysis and recombination, which may then be corrected for the effect of the destructive reactions. With the knowledge that the reversion is an equilibrium reaction, its effect may be minimized in a study of acid hydrolysis by selecting conditions conducive to low concentration of dextrose; *e.g.*, dilute solutions or starch in the early stages of commercial conversions. The effect of the several variables on each component part of the reaction may then be determined.

4. Hydrolysis Reaction. Freudenberg, Kuhn, and others (11-13) have proceeded on the assumption that the hydrolysis is a monomolecular reaction involving the scission of equivalent, 1-4 α -glucosidic bonds. They have applied a simple statistical treatment to predict values to be obtained for glucose, maltose, and higher homologues for any given stage in the early stages of the hydrolysis. The assumption should also be made that the few 1-6 α linkages (possibly 5% of the total) are hydrolyzed, if at all, under the conditions employed in the last phases of the conversion. From the equations developed, these workers have determined the rate at which any degradation product of any given length is formed and the maximum yield of this product which may be obtained during a hydrolysis of a polysaccharide chain. According to Freudenberg and Kuhn, the maximum yield, $\phi_{\max.}$, as a fraction of the starting material, is equal to the number of chains of length n , Z_n , times n units per chain, divided by N , the total number of units.

$$= \frac{nZ_{n \max.}}{N} = n \left(\frac{2}{n+1} \right)^2 \left(\frac{n-1}{n+1} \right)^{(n-1)}$$

Calculations for monose, biose, triose, and tetrose are given in Table XXV. The theoretical course of the reaction is shown diagrammatically in Fig. 83.

TABLE XXV
Application of Statistical Method to Starch Hydrolysis

Fragment	Maximum yield at			Maximum yield	Hydrolysis at maximum yield
	25% hydrolysis	50% hydrolysis	75% hydrolysis		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Monose.....	6.3	25.0	56.2	100.0	100.0
Biose.....	9.4	25.0	28.1	29.8	66.7
Triose.....	10.5	18.8	10.5	18.8	50.0
Tetrose.....	10.5	12.5	3.2	13.8	40.0

To verify these results for starch by experiment, however, requires better analytical methods than the determination of total reducing sugars by Fehling's

test, polarimetric measurements, diastatic analyses, or similar approximations which have been applied in earlier work. Cantor and Moyer (14) have discussed the development of applicable analytical methods, especially those which have been reported recently. These include the method of Sichert and Bleyer (15) to estimate dextrose by use of a modified Barfoed reagent (copper sulfate and sodium acetate), the method of Hurd and Cantor (16) to estimate the lower sugars by methylation and fractional distillation, the method of Hurd, Liggett, and Gordon (17-19) by which the lower sugars are converted to propionic esters and are fractionally distilled, and a modified method of the A. O. A. C. for the determination of dextrans by alcoholic precipitation and correction for the reducing substances in the precipitate.

Cantor and Moyer have discussed the accuracy of the above methods and have compared the results of analyses of sirups representing various stages in the acid hydrolysis of corn starch. The percentage composition of conversion liquors over the range of 25 to 60% hydrolysis is shown in Fig. 84. The assumption is made that the total reducing value by Fehling's test, calculated as per cent dextrose (dextrose equivalent, D. E.), expresses the degree of hydrolysis. In Table XXVI the results of analysis of a corn starch sirup at 42 D. E. are compared

TABLE XXVI

Comparison of Statistical and Actual Analyses of Acid-Modified Corn Syrup

Constituent	Statistical analysis per cent	Actual analysis per cent
Dextrose	17.7	22.1
Maltose	20.6	20.8
Maltotriose	17.9	15.3
Maltotetrose	13.3	4.5

with the results of statistical analyses. From a comparison of these values and from a comparison of other values given in Figs. 83 and 84, it will be seen that only in the case of maltose is there an agreement between found and calculated values. The percentage of dextrose found is decidedly higher than was anticipated, and the amounts of higher sugars are considerably lower than the predicted ones. It is obvious, therefore, that the course of starch hydrolysis cannot be considered as a random hydrolysis of equivalent glucosidic linkages even in the early part of the reaction and that the suggested course of the hydrolysis is an oversimplification of the actual manner in which starch is degraded.

Inasmuch as the reaction rate for maltose is higher than the average rate for starch, Cantor and Moyer have proposed that a terminal dextrose unit is more easily hydrolyzed in order to account for the higher amount of dextrose found. This supposition would seem logical. However, it would also seem probable that any maltose which is formed in the course of hydrolysis would, for this same reason, be more speedily hydrolyzed to dextrose than the rate at which more maltose is formed by random rupture of glucosidic bonds. The amount of maltose present at any given time consequently should be decidedly less than

would be anticipated from calculations by the statistical method. Therefore, the agreement between the per cent of maltose found by analysis and that predicted is not significant, but rather must be fortuitous. It might also be recalled that the fact that the rate of hydrolysis of maltose is higher than the

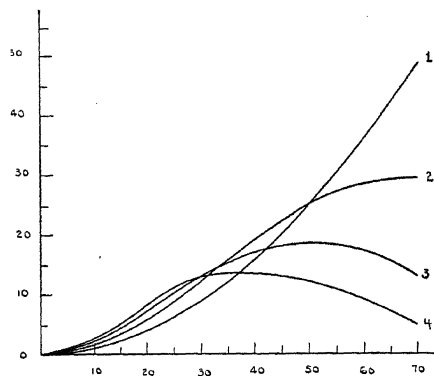


FIG. 83. Theoretical course of starch hydrolysis by statistical analysis. Curve 1, monose produced; Curve 2, biose produced; Curve 3, triose produced; Curve 4, tetrose produced. Abscissa, per cent degradation; ordinate, per cent yield.

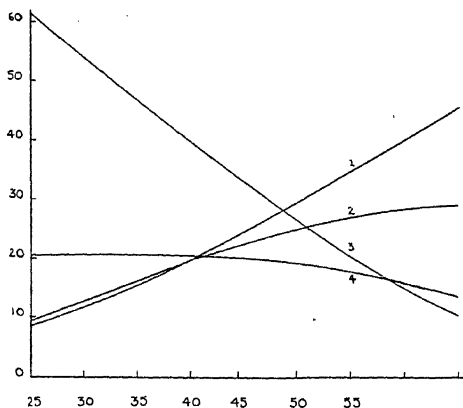


FIG. 84. Percentage composition of starch conversion liquors. Curve 1, dextrose; Curve 2, maltose; Curve 3, dextrins; Curve 4, higher sugars. Abscissa, dextrose equivalent; ordinate, per cent composition.

rate for starch led to the supposition which prevailed for several years in the industry that corn sirups contain very little maltose. The issue was settled by Berlin (20) who demonstrated the presence of substantial quantities of this sugar in these sirups.

Possibly some error is involved in the study of the kinetics of the reaction by using copper reduction values calculated as percentage of dextrose (D.E.) as a

direct measure of the percentage of glucosidic bonds hydrolyzed in starch. In particular, the method appears to be unsuitable to detect the extent of hydrolysis where a scission is made which results in an aldehyde group attached to a long chain of glucopyranose units (a so called, non-reducing dextrin). Rather, the results of the Fehling's test give greater emphasis to the proportion of shorter chains which may be present in the hydrolysis mixture at any given time. From the work reported by Levine, Foster, and Hixon (4) it would appear that every scission which results in the formation of an aldehyde group, whether the latter is a part of a short or relatively long chain, can be correctly determined by oxidation with iodine. Sadovy (21) has followed the acid hydrolysis of starch by measuring the amount of liberated aldehyde groups iodometrically.

Applying the monomolecular reaction equation to his results, Sadovy finds that the reaction constant decreases during the reaction. Taking an average of the values for K during the early part of the reaction and using the equation

$$K = \frac{2.3(2 - \log(100 - Z))}{Ct}$$

where Z is the hydrolysis product, expressed as per cent of total dry substance, C is the volume per cent acid, and t is the time in minutes no difference in the average value was found for a change in the starch concentration. A value of K equal to 0.47 was found when 12% of dry substance (by volume) was used in the hydrolysis; a value for K equal to 0.48 was found when 22.3% dry substance was used. Quite obviously, had the starch concentration been increased to very high values, for example to double the concentration investigated, it would be expected that the reaction constant would have decreased owing to the effect of a recombination of the dextrose, as mentioned previously.

The temperature of hydrolysis used by Sadovy was 133° C. and an acidity of 0.1% HCl (by volume) was employed. With the same acidity, the value of K was found to vary with a change in the temperature of hydrolysis according to the equation

$$\log K = 0.0418t - 5.884$$

The determined values for K at various temperatures are compared with those calculated for K according to the above equation in Table XXVII. It will be seen that each 10° C. rise in temperature results in very nearly a 3-fold increase

TABLE XXVII

Actual and Calculated Values for Starch Hydrolysis. Reaction Rate Constant at Various Temperatures of Hydrolysis

Hydrolysis temperature ° C.	K by experiment	K by equation
119	0.125	0.123
133	0.470	0.473
138	0.770	0.766
143	1.200	1.230

in the value for K . The critical increment, E , may be calculated by means of the Arrhenius equation

$$\lg \frac{K_2}{K_1}$$

where K_2 and K_1 are the reaction rate constants at the absolute temperatures, T_2 and T_1 , respectively and R is the gas constant, 1.98 cal. E is found to be 31,000 cal.

Within a limited range of hydrochloric acid concentration, Sadovy finds that the rate of hydrolysis is proportional to the acid added, expressed as per cent by volume. Quite naturally, however, this relationship could not be expected to hold at extreme acid concentrations. The acid-binding power of the starch, to be discussed in a following section, would materially lower the acid activity at extreme dilutions. At high acid concentrations, particularly if the less ionized acids are used, the acid activity would not be proportional to the amount of acid added.

5. Recombination. The rate of glucose recombination, or reversion as it is frequently called, has been studied by Moelwyn-Hughes (22). The reaction of 10, 20, 30, 40, and 50% dextrose solutions, in normal HCl at 60° and 70° C., was followed polarimetrically. This investigator found that the reaction proceeded with a progressive decrease in the dextrose content until an equilibrium was reached in each case. Both the position of equilibrium and the rate of the reaction in approaching this equilibrium are independent of the initial dextrose concentration. The critical increment for the reaction is about 33,500 cal., which is substantially higher than the value for starch hydrolysis. Hence the recombination reaction increases more rapidly with rising temperature than the hydrolysis reaction. High converting temperatures are, therefore, to be avoided, or otherwise the yield of dextrose will suffer accordingly.

More recently, Silin and Sapedina (8) have investigated the recombination reaction and have determined the equilibrium point for many sets of experimental conditions. From their data, they have developed a modified bimolecular equation for the reaction

$$K = \frac{An^2}{100(100 - n)}$$

where K is the reaction constant, A is the initial glucose concentration in the percentage by weight of the water present, and n is the glucose remaining at equilibrium as the percentage of the initial amount. The constant, K , is found to be 259 over the range of 8.6 to 62.9% for the initial glucose concentration (or 9.4 to 170% by weight of water present). If the determined value is substituted for K and n is calculated, the percentage of dextrose remaining at equilibrium for any initial dextrose concentration is given by the equation

$$n = \frac{100(\sqrt{1 + 0.015444A} - 1)}{0.007722A}$$

Table XXVIII gives a series of dextrose equilibria values. It is to be noted that

TABLE XXVIII

Dextrose Reversion Equilibria at 100° C.

Initial concentration of starch, per cent by weight	Initial concentration, based on dextrose		Per cent dextrose (dry basis) at equilibrium
	Per cent weight of mass	Per cent weight of water	
0.0	0.0	0.00	100.0
4.5	5	5.26	98.1
9.0	10	11.11	96.0
13.5	15	17.65	94.0
18.0	20	25.0	91.8
22.5	25	33.3	89.6
27.0	30	42.9	87.4
36.0	40	66.7	82.5
45.0	50	100.0	77.1
54.0	60	150.0	70.9
63.0	70	233.3	63.6

at a dextrose concentration of 20% (20 g. of dextrose in 100 cc. of solution) only about 92% of the dextrose remains at equilibrium. In hydrolysis it is evident that the use of starch of high concentration results in reduced dextrose yields; whereas the lower the starch concentration used, the higher will be the final yield of dextrose.

The practical aspects of these observations are emphasized by Silin and Sapedina. They point out that in starch conversion concentrations which should lead to a final dextrose concentration of 25% at the end of the hydrolysis, result in the production of 89.6% of this amount of dextrose. The remaining 10.4% consists principally of the products of reversion (such as gentiobiose). After crystallization of the dextrose, the "hydrol" (mother liquor) contains this 10.4%. Since the "purity" of "hydrol" in respect to dextrose is 66.7%, according to these workers twice as much dextrose is present in "hydrol" as the total products of reversion. The 10.4% of the latter prevents the crystallization of 20.8% of dextrose. Therefore, not 10.4%, but $10.4\% + 20.8\% = 31.2\%$, or nearly a third of the expected dextrose yield, is lost owing to the total effects of the recombination reaction.

Silin and Sapedina point out that instead of a reconversion of "hydrol" to recover a part of this dextrose loss, it would be more practical to resort to a lower starch concentration in the initial conversion. Reconversion requires dilution and the addition of more acid and more sodium carbonate. The added water is as expensive to evaporate after reconversion as after the primary conversion. The addition of acid and sodium carbonate materially increases the sodium chloride content of the reconverted "hydrol" and salts of this type are known to affect the crystallization of dextrose adversely. These investigators propose the use of a starch concentration which will result in a 15% solution of

dextrose, if the yield was 100% of the theoretical. At this concentration, the purity of the sirup is 94% (as dextrose) at equilibrium, only 6% being lost in recombination. The "hydrol" therefore contains $6\% + 12\% = 18\%$ of solids to be considered as a loss of dextrose. Assuming that a 25% concentration (as dextrose) represents normal conversion, a reduction of the concentration to 15% results in an increase in the dextrose yield of 13.2% above normal.

It should be pointed out that the 25% concentration is not commonly used in America for dextrose production. For many years starch suspensions of 12° Bé. have been used which have a solids content in the neighborhood of 18%, allowances being made for a chemical gain in water during hydrolysis and for the dilution caused by the condensation of the steam used to supply the heat for the reaction. Experiments performed by W. B. Newkirk and his associates have shown that this concentration is the optimum in respect to the economy of the process; that is, when the cost of a larger volume of water to evaporate (owing to higher dilution) and other factors are balanced against the value of the increased yield of dextrose which results from the use of a low concentration of starch.

The "hydrol," or mother liquor from dextrose crystallization, has been extensively examined in order to shed further light on the nature of the complex reaction which results when starch is hydrolyzed with acid. Miller (23) describes "hydrol" as a dark colored, viscous sirup containing 78.3% solids and 21.7% water. The solids contain 7.93% ash. Hurd and Cantor (16) report the analysis of "hydrol" in respect to sugars as 40.1% monosaccharides, 28.5% disaccharides, and 4.0% trisaccharides. The analysis was performed by the methylation and fractional distillation of the component sugars. Miller (23) succeeded in obtaining the acetate of gentiobiose directly from "hydrol," whereas Berlin first rid the "hydrol" of fermentable sugars by the action of yeast. Among the non-fermentable sugars, Miller found a substantial quantity of another disaccharide in addition to gentiobiose. He has prepared derivatives of this sugar and has described their properties. It may be the same sugar as the isomer of gentiobiose found in "hydrol" by Coleman, Buchanan, and Paul (24). The presence of 6-(α -glucosyl)-glucose in "hydrol" could, moreover, be accounted for as an incompletely hydrolyzed residue from the amylopectin fraction of starch.

6. Intramolecular Dehydration and Other Destructive Reactions. The destructive or irreversible reactions into which dextrose enters in the presence of acid have received very little quantitative study. The actual loss in yield which results from the formation of products of the furfural and levulinic acid class is relatively small under the conditions normally used in sirup and dextrose manufacture. Means of measuring with accuracy very small concentrations of these products have been lacking. The undesirability of these side reactions in practice is due more to their reflection on the quality than on the quantity of the primary products.

The production of levulinic acid is slight during the time allowed for conversion of the starch to sirup or sugar. This is evident from the data submitted

Recent patents issued to Thompson (25) and to Moyer (26) for the production of this acid from starch or starch products. Thompson suggests treating 200 g. of carbohydrate in 400 cc. of water with enough HCl to give a concentration of 5% hydrochloric acid and heating at the boiling point of water, under a reflux, for 22 hrs. The hydrochloric acid, together with the formic acid which is formed simultaneously with the levulinic acid, is distilled off, and the levulinic acid is recovered after a vacuum distillation. The use of "hydrol" is recommended as a substrate for this destructive reaction. Not only would "hydrol" seem a logical choice for reasons of economy, but also because it might be concluded that it already contains some levulinic acid as a result of the primary acid treatment of the starch from which the "hydrol" is produced. Thompson also found "hydrol" suitable for use in that it contains electrolytes which increase the activity of the added HCl. From 265 g. of "hydrol," 48 g. of levulinic acid were recovered. The yield of levulinic acid was found to vary inversely with the concentration of "hydrol" in the solution treated. Yields of levulinic acid as high as 35%, based on the total dry substance, are reported when the "hydrol" concentration is reduced to 19 g. in 400 cc. of water.

Moyer suggests a treatment in which starch and HCl are preheated to 100° C. at atmospheric pressure in an initial step. The strength of HCl used is between .5 and 3.5%. Starch is used in such a concentration that a molar ratio of hydrogen chloride to dextrose units is maintained at 0.15 to 0.25. Suspensions of the starch of 18.5° Bé. are recommended for the process. After the preliminary digestion, the liquors are transferred to a pressure cooker where the temperature of the reaction is slowly raised to between 175° and 215° C., after which the reaction mixture is held at 190–200° C. until the desired results are obtained. A controlled method, such as by the introduction of live steam, is suggested for raising the temperature from 100° to 200° C. so that the rate of heating may be held to a temperature rise of about 2° C. per minute. Faster heating unduly extends the dextrose destruction beyond the levulinic acid stage to the point where polymerization, leading to the formation of humin, is excessive. The production of non-condensable gases also attends humin formation.

The disappearance of dextrose is followed polarimetrically, and the time is noted when 99% of the substrate has reacted. Levulinic acid does not immediately appear in proportion to the amount of dextrose which has reacted. This result substantiates the conclusion that the acid forms through an intermediate reaction, quite possibly the formation of hydroxymethyl furfural. Therefore, the heating period is extended twice as long as the time required for 99% of the initial dextrose to disappear from the solution. Moyer treats 162 lbs. of starch suspended in water at a density of 18.5° Bé. by adding 32 lbs. of 28% HCl and raising the temperature from 100° to 200° C. over a period of 50 min. It requires about 25 min. at 200° C. to produce a yield of 56 lbs. of levulinic acid. This is 8.5% of the theoretical yield. The total reaction time is 50 plus 25 which equals 75 min. Formic acid, produced simultaneously with the levulinic acid,

is removed by distillation after partial neutralization of the liquors to pH 1.9 with sodium carbonate.

If we assume that levulinic and formic acids originate from hydroxymethylfurfural, it would seem reasonable to suppose that both form in equimolar proportions.¹ According to these assumptions, the amount of each produced in the acid hydrolysis of starch may be estimated from the difference between the total acid present after conversion, determined by potentiometric titration, and the amount of hydrochloric acid added.

Techniques to measure quantitatively the amounts of the precursor of these acids at different points in the acid hydrolysis of starch have been lacking. An advance was made in this phase of the study, therefore, by the observation of Cantor and Peniston (28) that hydroxymethylfurfural may be determined by the use of the polarograph. These workers found that this substance is reducible at the dropping mercury electrode. The half wave potential is sufficiently removed from that observed for dextrose and maltose by Kerr (29) that measurements of the wave height offer a good method for quantitative estimation in starch hydrolysates. Hydroxymethylfurfural has been shown to be present in "hydrol" by Cantor and Molteni [quoted by Miller (23)] by this method.

The extent of the destructive reactions may now be determined by taking the sum of the amount of hydroxymethylfurfural found by the polarographic method and the amounts of levulinic and formic acids estimated by titration.

No published data on the rate of hydroxymethylfurfural formation are as yet available. However, from private communications with the originators of the polarographic technique to measure this quantity it is known that the critical increment in the temperature range of 120–142° C. is quite close to that for the recombination of dextrose. Hence the rate of hydroxymethylfurfural formation, as is also the case for the recombination reaction, is increased more in proportion than the hydrolysis reaction as the temperature is raised. The rate of the destructive reaction is proportional to the effective acid concentration.

Sadovy (21) has estimated the combined effects of the recombination reaction and of the destructive reactions by a determination of the loss in dextrose which results when pure dextrose solutions are heated with acid under fixed conditions. 20% solutions to which 0.1% of HCl (by volume) was added were heated at 143° C. for various periods of time. When starch is treated with acid, under comparable conditions, it is found that the amount of dextrose present during the latter stages of the reaction is less than is estimated by applying a correction for dextrose reversion and destruction obtained according to the manner indicated above. For example, after a heating period of 45 min., Sadovy found that dextrose solutions have decreased only 3% in their dextrose content. After a comparable treatment of starch, the dextrose content of the reaction mixture indicates that about 8% of the dextrose produced has either recombined or has been destroyed. Sadovy explained the discrepancy by assuming that a part of

¹ The work of Teunissen (27) justifies this assumption.

the starch does not undergo hydrolysis. At least it would seem plausible to conclude that many of the glucosidic bonds in starch do not hydrolyze at the rate anticipated.

In summation, therefore, it may be stated that even though corrections are made for the effects of secondary reactions, the reaction rate for the hydrolysis of starch is greater in the initial stages, then equals, and finally becomes less than that calculated by means of a monomolecular reaction equation for a strictly random hydrolysis of equivalent linkages. The discrepancies will be explained, no doubt, when the problem is approached with due regard for the physical chemistry involved, as for example by recognizing the several possibilities: (a) that the hydrolysis observed is a composite of at least two reactions, the splitting of comparatively short linear chains of glucopyranose units and the splitting of much larger branched structures; (b) that some glucosidic bonds are preferentially hydrolyzed owing to physical forces which place them under strain; (c) that not all of the glucosidic linkages are equally available to the activity of the acid owing to the ability of certain component parts of the starch to orient into resistant complexes; and (d) that there are types of glucosidic linkages present in starch components which no doubt require a higher activation energy than that for the normal or predominating type. The more recent advances in starch chemistry, described in previous chapters on the physical and chemical properties of the starches, should make the problem of starch hydrolysis approachable by a logical plan of investigation, and the kinetics of this complex reaction should shortly become known.

7. Effect of the Substrate Starch on the Acid Concentration. The effect of the starch on the concentration of the active acid during hydrolysis has been the subject of many researches. It has been known for many years that the acid activity in hydrolysis mixtures containing starch is not proportional to the amount of added acid but that it is usually a value less than this amount. Attempts have been made to explain this discrepancy by assuming that the starch, some part of it, or some impurity associated with the starch either combines with the acid or acts as a buffer. Since the rate of hydrolysis is proportional to the acid activity, the study of methods to purify or prepare the starch for hydrolysis assumes importance because variable acidities lead to variable results and possibly to losses in yield. Such treatment of starch might be one of additional refining or purification from interfering impurities or, when this is not possible, of the production of an "acid starch" with a buffer capacity much less than that of the untreated starch.

One of the earlier investigators of the problem, Silin (30), found that as much as 40% of the sulfuric acid added to hydrolyze potato starch may be bound by this substrate. Widmeyer and Rall (31) studied the effect and, expressing their results on a basis of the percentage of starch used, found that corn starch binds between 0.022 and 0.087% of HCl, while potato starch binds 0.052%. Smirnov (32) has recently reviewed the subject. He has pointed out the acid-binding capacity of impurities and other residues associated with the starch. Among

those considered are proteins, fats, cellulose-like material, SiO_2 , phosphates, and other salts of Ca, Mg, K, and Na. Of these, nitrogenous and phosphoric acid residues appear to exert the greatest buffering effect, particularly the type of protein which might be associated with potato starch. However, no constant figure is arrived at for the acid-binding power of starch, either of corn or potato since the composition of each type varies with the different samples tested. Of twelve samples analyzed and inspected for acid-binding power, however, it was found that this power could be correlated with starch composition in respect to the two residues mentioned. Although the basic constituents of the ash might be expected to exert a certain amount of acid-binding, it is pointed out that the composition varies widely in respect to these constituents. This difference in ash content is to be expected inasmuch as starch is a fairly effective adsorbent and inasmuch as different mills show considerable variations in the composition of their various mill liquors. This is particularly true in respect to the cations present. For example, starch-producing mills which are also engaged in glucose production frequently use either softened water or steam condensate or a combination of the two as a source of water for their starch-milling and converting processes. The natural Mg, K, and Ca ions associated with native starch are therefore being continuously removed throughout the milling process or being continuously replaced with sodium ions as the starch comes in contact with liquors progressively higher in sodium ion content. Samec (33) has adequately discussed the adsorptive capacity of starch in respect to metallic ions and ammonia. The practical result of the replacement of the natural cations of starch by sodium ions is that starch sirups made from the hydrolysis liquors of such starch show decidedly less tendency to become turbid as the result of the formation of insoluble sulfates. (The sulfate ion arises from the sulfite used for the bacteriological control of the wet milling processes.) The nitrogenous material adsorbed on starch, some types of which appear to accelerate the formation of color during and after hydrolysis, likewise varies from starch to starch, particularly in respect to the class of nitrogenous substance. This difference also results, in part, from variations in the composition of process waters employed in milling, owing to fluctuations in the control of their biological activity.

In the case of some starches, potato, for example, most of the phosphate found with the starch is esterified with constituents of branched configuration the amylopectins. Samec (33) has shown that this acid phosphate group is liberated in the very early stages of hydrolysis but even though it were not, the amylopectin acid phosphate should exert a measurable amount of buffering action. The chemically bound phosphate in corn starch is, no doubt, too insignificant in amount to account for or to be considered in calculations of the acid-binding power of the starch. The buffer in this case is undoubtedly principally nitrogenous in character. In corn starch mills producing sirups and dextrose, the remainder of the acid-binding power of the starch may be attributed to the sodium salts of weak acids, such as phosphoric and lactic, which have been

adsorbed by the starch during milling. Acid phosphate arises from the hydrolysis of phytin, the phosphorus reserve of the corn kernel.

Because of the wide variation found in respect to starch composition, Smirnov recommends that the acid-binding power of the starch used in hydrolysis be determined periodically. Two methods are suggested. The first method was originated by Silin (30) who calculated the amount of bound acid from the deviation in the anticipated proportionate increase in the rate of hydrolysis compared with the increase in acid concentration. The method is as follows: 30 g. of starch (dry basis) and 50 cc. of water are preheated to 55° C. for 15 min. Based on the weight of starch to be treated, 0.182% of *N* HCl is prepared in 100 cc. of water. The acid is boiled, and the preheated starch suspension is added gradually over a period of 5 min. The mixture is made up to 250 cc. and then boiled under a reflux. The hydrolysis is followed by an iodometric analysis for reducing groups. A duplicate hydrolysis is carried out with the same starch with 0.364% of acid. Assuming that the starch contains impurities which bind $x\%$ of acid, the time required to reach a certain limiting value in the hydrolysis for the lesser amount of acid, T_1 , and for the larger amount of acid, T_2 , being known, then

$$\frac{0.364 - x}{0.182 - x} = \frac{T_1}{T_2}$$

Solving for x gives the amount of acid bound as per cent of dry starch.

It would seem that this method or an elaboration of it possesses particular merit for several reasons. First, not only is the effect of starch residues which reduce the acid activity determined, but the result is corrected for the effect of increased acid activity exerted, possibly, either by the carbohydrate itself or substances associated with the starch. Secondly, the determination is made, or can be made, at the elevated temperature used in hydrolysis, and hence the influence of the buffers is determined on the actual acid activity at this elevated temperature. Both of these, the buffering effect and the acid activity before buffering, are probably different at lower temperatures from what they are at higher temperatures.

The effect of electrolytes, in particular NaCl, in increasing the acid activity when carbohydrates are treated with acid has been noted by several workers including Thompson (25). The amount of such electrolytes as NaCl present in the starch is therefore important in practice for the prediction of the rate of hydrolysis, for a given addition of acid. The method of Silin for estimating the effect of the substrate on the acid activity of a given addition of acid should take into account and correct for the possible "salt effect" of the starch used.

The second method used by Smirnov for estimating the acid-binding capacity of starch was apparently suggested by Widmeyer and Rall (31). The hydrolysis mixture of starch and acid is prepared as in the first method. It is then autoclaved for 7 to 8 min., cooled to room temperature, and enough water is added to make the total weight 450 g. The solution is titrated potentiometrically with 0.1 *N* NaOH. The type of titration curve obtained is shown in Fig. 85. An

inflection may be noted in the neighborhood of pH 4.5. Hence, the difference in the amount of acid added to the starch and the amount corresponding to the alkali used to titrate the hydrolysate to pH 4.5 is taken as the amount of acid bound by the starch.

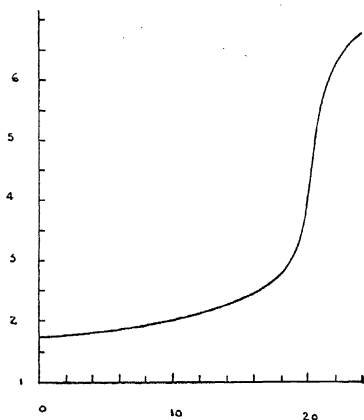


Fig. 85. Potentiometric titration of hydrolyzed starch. Abscissa, cc. 0.1 N NaOH; ordinate, pH .

Although the use of these methods for the determination of the effect of the substrate on the acid activity affords a means to estimate the true acid activity in starch hydrolysis, their use in practice has certain limitations. Not only will the acid-binding power of starch be found to vary with the type of starch, *i.e.* corn and potato, and with the starch-milling process employed, but even within a mill the starch made will be found to vary from time to time with different batches of starch. This requires that each batch of starch be tested and the results determined before it is hydrolyzed. It would seem more logical to develop better means for pretreating the starch in manufacture to secure a purification from or neutralization of interfering constituents as far as possible.

8. Neutralization and Clarification. In the manufacture of sirups and sugars from the hydrolysates of starch, neutralization of the acid used is commonly accomplished by the addition of sodium carbonate. Following this step, the liquors are clarified by filtration to remove suspensoids and other colloidal material including certain colored bodies. In earlier processes the liquors were allowed to settle, they were passed over vacuum filters, sometimes precoated or they were submitted to both procedures. Bone char filters were and are used for final clarification. Apparently the two steps of the manufacturing process, neutralization and clarification, are intimately connected in some respects.

Liquors from an extended acid hydrolysis of corn starch, such as those employed for dextrose manufacture, contain as insoluble or readily precipitable material small amounts of fatty acids (34), protein bodies, and lesser amounts of

fiber and inorganic constituents. A less extensive hydrolysis, such as is used in the production of various sirups, results also in the presence of small amounts of carbohydrate material, sometimes referred to as insoluble dextrins. Quite likely this material represents the unconverted residues from a part of the amylose fraction of the starch. Paine and Badollet (35) observed that the flocculation of colloids after starch hydrolysis is influenced by the *pH* to which the liquors are neutralized. They suggested the use of *pH* control in the neutralization in order to arrive at what appears to be an isoelectric point for the coagulum and hence to obtain the precipitation of a maximal amount of this material. They have also noted that the character of the precipitate obtained in a sugar conversion varies in some respects from that obtained from a conversion to produce a sirup. For example, the isoelectric point is slightly different in the two cases, being about *pH* 5.0 for the former and 4.5 for the latter. In another publication Badollet and Paine (36) emphasized the point that, if sodium carbonate is added to starch hydrolysates in excess of the amount necessary to adjust the *pH* to the isoelectric point, a part at least of the suspensoids redissolves, thus placing an undue load on the bone char filters. Inasmuch as the colloidal material present possesses a positive charge, these workers have proposed the use of such adsorbents as aluminates, aluminum silicate, and, particularly, bentonite, which have a negative charge at the *pH* employed for the clarification of starch hydrolysates. The bentonite may be used for clarification directly after the acid conversion. Its use apparently possesses the advantage over the use of sodium carbonate in that a slight excess of the clay does not redisperse the colloidal impurities in the liquor. Badollet and Paine reported the satisfactory use in practice of sodium aluminate for clarification, the aluminate being added in such amounts as to bring the *pH* of the converted liquors to the isoelectric point.

The uncontrolled use of sodium carbonate for neutralization possesses another distinct disadvantage in that glucose solutions develop color at an accelerated rate if the *pH* is raised to 5 or above, particularly when they are treated at elevated temperatures, as has been pointed out by Sjostrom (37). This effect has been studied in further detail by Fellars, Millar, and Onsdorff (38) and by Kroner and Kothe (39). The latter find that a sharp minimum in the formation of color occurs at *pH* 2.3 to 3.0 when glucose solutions are heated. This added color again increases the burden imposed on the bone char filters or, stated otherwise, requires an excessive ratio of a weight of bone char to clarify a given amount of sugar solution. Moreover, the efficiency of the bone char used in refining is related to the *pH* of the sugar solution; most chars appear to be very efficient in the *pH* range in which the formation of additional color is accelerated.

An additional refining problem which develops from the use of acid in the hydrolysis and subsequent neutralization is that the salt so formed may adversely affect the quality and yield of the product. The early use of lime for neutralization of sulfuric acid led to the formation of gypsum which, if not removed from solution below a value represented by the solubility product constant of the ions, resulted in sirups which developed a haze of gypsum on standing. The

more recent use of sodium carbonate to neutralize hydrochloric acid leads to the formation of sodium chloride. The presence of this salt, in amounts equivalent to that in liquors from dextrose crystallization has been pointed out to affect the crystallization of the sugar adversely (8, 40). The salt exerts a solubilizing effect on the sugar.

The problems outlined have instigated an intensive search for more suitable carbons and other types of adsorbents for use in sugar refining, for better methods to utilize these adjuncts, and a review of the problem of the neutralization of glucose solutions. In an early patent Duryea (41) outlined a process for treating starch hydrolysates with 1 part of tannin to every 4800 parts of liquor, heating to 90° C., and filtering. Meisel (42) suggested the use of zeolites for the clarification of starch hydrolysates, claiming the advantage that all metals below sodium in the electromotive series (such as calcium and iron) are removed. A more recent patent application by Pfeiffer and Langen (43) recommended the use of zeoliths to clarify sugar liquors, and pointed out that all cations may be replaced with the hydrogen ion. Smitt (44) also proposed the use of cation exchange on glucose liquors by the use of carbonaceous zeolites. These are prepared from sawdust by carbonizing with sulfuric acid. This worker suggested a preliminary neutralization and filtration before exchange of the cations. The carbonaceous zeolites may, however, be added directly to the converter. An increased efficiency for color removal as compared to the usual bone char is noted for this specially prepared carbon. Both the ion exchange and the superior color-removing properties of this material from sawdust are attributed to a special physical structure in the adsorbent which does not develop in the carbons previously used.

Adams and Holmes (45) proposed use of a cation exchange on glucose liquors followed by an anion exchange. A resin prepared by treating tannins with formaldehyde is used for cation exchange, and a resin prepared by condensing formaldehyde with aromatic amines (46) removes the acid. The possibility of preparing sugar solutions free from both cations and anions, leaving the liquors substantially electrolyte-free, has been pointed out in detail by Liebknecht (40, 47, 48). Carbonaceous zeolites are prepared to remove cations and keratiniferous material is used to remove the anions. The regeneration of the ion exchanger, so that it may be used in successive cycles, is also discussed. The advantage of preparing electrolyte-free sugar solutions, in that the adverse influence of salt on the crystallization of the sugar is minimized, is pointed out by Liebknecht.

Boyd (49) suggested pretreatment of the glucose liquors from the starch hydrolysis with diatomaceous earth in order to obtain a preliminary clarification before the treatment for ion exchange. Apparently the type of resins suited for use in this operation is not limited, as is evidenced by the voluminous literature on the subject. Boyd suggested the use of a resin prepared from catechol tannins by treatment with oxalic acid, sulfur, or alkaline sulfides. This resin may be regenerated, after use to remove the acid from dextrose liquors, for

treating successive batches of the sugar. Swain (50) proposed the use of biguanidine formaldehyde resins for anion exchange in sugar solutions.

Obviously, the resins and other ion exchangers used or suggested should retain their efficiency and should be relatively stable at the temperature and for the other conditions employed in the refining of starch hydrolysates. When and if these characteristics are obtained or developed, it is to be expected that the fundamental knowledge which is being collected in this field of study will be applied to good advantage in the refining of glucose solutions. Not only is their use indicated in the primary refining operations but it would seem that the use of ion exchangers is equally applicable in the further treatment of secondary products, such as the mother liquors of dextrose crystallization, in operations to increase the yield of sugar obtainable.

9. Secondary Catalysts. One might be led to suspect from the foregoing discussion that, if the conditions for starch hydrolysis by acid were held within a given limit of variation, the course of the hydrolysis would be practically identical in each case. It might be expected that the distribution in the percentage of the different lower saccharides at any given point in the hydrolysis would always be very nearly the same and, conversely, if a product were analyzed for its content of various sugars, such as glucose, maltose, and their homologues, one might be able to conclude whether or not the sirup were made by acid hydrolysis. Such, however, is not the case.

There is a measurable difference in the course of hydrolysis between that obtained with hydrochloric acid and with sulfuric acid when both are used in comparable amounts. Quite recently a more striking variation in the normal course of hydrolysis, with a given acid such as hydrochloric, has been reported by Langlois (51). This effect is obtained by inclusion in the hydrolysis mixture of very small amounts of metallic substances. For example, Langlois reported that the addition of 0.013% of molybdenum, based on the weight of starch present, resulted in a sirup (at 70 to 88% reducing sugars, estimated as dextrose) which has a decidedly lower specific rotation, a higher percentage of fermentable sugars, and which shows less tendency to crystallize, than a similar hydrolysate (same total reducing sugar content) prepared without the secondary catalyst. Indeed, the specific rotation may be from 7° to 14° lower when molybdenum has been used than when it has not.

The molybdenum may be added to the usual type of conversion in the form of molybdic acid, compounds of molybdenum with halogens, and as the oxide of molybdenum. In an earlier communication, Langlois and Moyer (52) reported that similar effects may be obtained in starch hydrolysis by the use of other secondary catalysts such as aluminum, chromium, and tungsten.

The values reported for conversions when these secondary catalysts are used leads one to conclude that the results obtained in commercial conversions may be influenced, possibly in an undesirable manner in some instances, by the type of metal vessel used for carrying out the hydrolysis. On the other hand, the practical value of being able to produce a stable sirup which contains a very

high percentage of reducing sugars which are fermentable cannot be overlooked. Langlois and Moyer intimate, for example, that after the metal catalyst is removed from the conversion liquors, the refined sirup might be utilized to advantage by the fermentation industry. It would seem that the fundamental aspects of the phenomenon discussed merit further research to supply an explanation for the anomalous results obtained with metal catalysts in starch hydrolysis.

10. Commercial Methods for Acid Hydrolysis of Starch. In practice, starch is hydrolyzed by acid to produce the following general types of products: (a) sirups which have purities (reducing sugars expressed as per cent of dextrose) of from 25 to about 55 (the composition of these sirups of varying purity is given graphically in Fig. 84); (b) sirups which have purities up to 65 and higher and which possess a higher maltose equivalent than is shown by sirups which are made by the use of the common procedures for the acid hydrolysis of starch (this variation in result may be accomplished, for example, either by subjecting a starch which is partially converted with acid to the range of purities mentioned above in (a) to an additional conversion by enzyme, or by adding to the acid hydrolysis certain agents which alter the course of the reaction); (c) crude sugars which are of approximately the same composition in respect to dextrose, maltose, and dextrin as are the liquors when they leave the starch converter (the purities of these sugars are usually between 70 and 80); (d) refined dextrose hydrate; (e) refined anhydrous dextrose; (f) refined β -dextrose. The refined anhydrous dextrose is substantially pure α -D-glucose, the hydrate contains a molecule of water of crystallization, and the β -dextrose is substantially pure β -D-glucose.

The acid conversion procedures and the subsequent refining operations used may vary, depending on the nature of the product. In all cases, however, a starch slurry is used to charge a closed cylindrical copper vessel which may be as high as 20 ft. and as much as 6 ft. in diameter. Sirups of type (a) are made by acidifying a starch slurry at 20–22° Bé. with HCl to about pH 1.8 to 2.0 and pumping the slurry into the converter over a period of about 10 to 15 mi. The addition to the converter is graduated so that the starch will gelatinize and be converted progressively without the formation of large lumps of paste in the converter. A steam pressure of about 30 lbs. is applied, and these conditions normally bring about conversion of the starch in about 20 to 30 min. When the desired purity has been reached, the liquors are blown to a neutralizing tank where sodium carbonate is added to adjust the acidity to about pH 4.8. The suspended solids are now removed, in part at least. Fatty material from corn starch may be skimmed off in shallow tanks designed for the purpose, or it may be removed in fat-separating centrifuges. The liquors are passed to vacuum filters such as an Oliver or Sweetland press, which may be precoated with a so called filter aid. The liquors then pass to bone char filters to effect a primary removal of colored constituents after which the filtrate is evaporated to about 30° Bé. and carefully adjusted to the desired pH. The concentrated liquors are clarified again by passing over bone char filters or by mixing with an activated

carbon and filtering the mixture. Concentration of the sirup is then continued in a vacuum evaporator to a final value which is normally about 42° Bé. Depending on its use and "purity" the product is designated as confectioners' sirup, brewers' body sirup, etc. Also, the sirup may be blended with other ingredients to impart various flavors and other characteristics to make a table sirup for household use.

Procedures for producing other specialty sirups, as mentioned above in (b), have been described by Kerr and Schink (5), Kerr, Meisel, and Schink (53), Langlois (51), and Dale and Langlois (54). In order to reduce the extent of secondary reactions which follow when starch is hydrolyzed to a high purity with acid, Kerr and coworkers convert starch slurries at a density of 12° Bé. This may be done in a closed converter at a steam pressure of 30 lbs. The acid conversion is stopped when a purity of 57 is reached, and after an adjustment of the acidity to about pH 5, the liquors are further converted with a purified (malt) diastase until a final purity of about 64 to 65 is attained. Further refining of the sirup is done in accordance with the outline given above. The product is characterized by its maltose equivalent which is about 40%, whereas the maltose equivalent of a sirup converted to a purity of 64 by acid alone is only about 30%.

At about a purity of 60, the unpleasant taste which arises from secondary reactions when starch is treated with acid becomes noticeable, particularly if the recombination reaction is favored by the use of starch slurries of high density. These secondary effects, however, do not necessarily limit the use of the resulting product for many important industrial uses. When only acid has been used as the catalyst to convert starch to liquors with a purity of 60 and above, the concentrated sirup tends to crystallize, and the crystals are principally those of dextrose. Accordingly, as mentioned above in (c), starch may be converted by the procedures given for the acid conversion of starch to a sirup showing a purity of 70 to 80, clarified and concentrated, and then run into shallow pans to crystallize. The sirup sets to a solid mass on standing, which is dried and chipped by metal knives to make a product which can be conveniently packed in bags. These products are, depending on the purity, the "70" and "80" starch sugars (or corn sugar when corn starch is used for conversion) of commerce. These were also called glucose sugar in the trade but they are obviously not to be confused with the sugar, glucose, which is now marketed as refined dextrose.

For the manufacture of the three refined dextrose sugars mentioned, the starch conversion differs from that for sirup production principally by the use of a starch slurry of low density, for reasons already mentioned, and by the use of more acid and a higher steam pressure. It is desirable that the hydrolysis reaction proceed nearly to completion. Normally, starch slurries of 10–12° Bé. are adjusted with HCl to an acidity of about 1.5 and are converted by the use of 40 to 45 lbs. of steam pressure. When the desired end-point has been reached (usually at about a purity of 90 to 91 for considerations relating to the maximal yield of dextrose, as given in previous sections) the liquors are given a preliminary refining similar to that employed in sirup manufacture.

Newkirk (55-58) has described the processes by which the refined sirups are crystallized and has discussed the factors involved in the final purification of crystalline dextrose. The process for the production of dextrose hydrate, introduced to the industry by this worker, is briefly as follows: The sirup liquors, after concentration to about 40° Bé., are passed into large cylindrical metal vessels equipped with slowly moving agitators. These crystallizers are seeded with a portion of "second" sugar which is obtained from further hydrolysis and crystallization of the mother liquors from the first crystallization. The liquors are held at about 38° C., and a slow stirring is maintained for several days. The mass is then transferred to centrifuges which remove the mother liquor. After the crystals are washed free from mother liquor in the centrifuges, they are dried and have a purity of 99 to 100. The ease with which the crystalline dextrose is purified in the centrifuges depends to a large extent on the type and character of the crystal produced. The latter depends on the operation of the crystallizer, which has been fully described by Newkirk (57).

The mother liquor and washings are further treated to secure additional yields of sugar. The dextrose may be recrystallized to make a product for pharmaceutical uses by procedures described by Newkirk (58).

The physical chemistry involved in the production of the anhydrous form of dextrose, by a crystallization from a water solution, has been ably discussed by Newkirk (57) who describes the process for its production essentially as follows: The hydrate form, after leaving the centrifuges, is redissolved in water to give a solution of 28-30° Bé. Then 0.8% of an activated carbon such as Darco is added, and the mixture is treated at 160° F. for 30 min. The liquors are filtered through a Sweetland filter press and then through special filters until the filtrate is perfectly clear. The optically void liquors are delivered to an evaporator (called a "strike pan") at such a rate that, by the time the charge has reached graining conditions, about 15 to 20% of the volume of the finished batch is present. Seeding with crystals is not employed, since the initial graining serves this purpose. Supersaturation of the "grain" is reduced by the introduction of a relatively large charge of the liquor at 30° Bé. During the growing stage of the "strike," or batch of crystallized anhydrous dextrose, the mother liquor contains on the average about 82% of dry substance. When the pan is approximately full and is reduced to the proper concentration, heating is continued until the crystal formation has developed sufficiently. The charge in the evaporator may be "boiled on water." Supersaturation is reduced so that a false "grain" will not develop when the charge is dropped to a mixing box which supplies the centrifuges. After the crystals have been freed of mother liquor, they are washed with hot water in the centrifuge in order to prevent chilling the residual mother liquor to a temperature favorable for the formation of the hydrate crystal.

A more recent product to be developed is the β -dextrose, mentioned under (f) above. The work of Newkirk (57) indicates the conditions under which this isomer forms in the crystallization of anhydrous dextrose by the procedures already outlined. The processes for producing the two anhydrous isomers are

very nearly the same except that in making the "strike" conditions are maintained (temperature and concentration) to favor the crystallization of the β isomer. A technically important characteristic of the β -dextrose is its extreme ease of solution in water. The product is used to a large extent by beverage manufacturers and for other applications in which the rate of solution is important from a manufacturing standpoint.

Other special sugars are produced in some volume. One of these may be mentioned, since it is sold extensively to certain industries which utilize the ability of the anhydrous dextrose to hydrate and therefore to "bind water." It may be referred to as quick hydrating anhydrous dextrose, and it is distinguished from the normal variety by the shorter length of time required (about 10% of the normal time) for the crystals to hydrate when they are mixed with a limited amount of water or an aqueous solvent. Apparently the explanation for the difference is that a very small amount of microscopically small hydrate crystals on the face of the anhydrous crystal induces the latter to change to the hydrated form at an accelerated rate. The writer observed that the change from the normal to the quick hydrating type of anhydrous dextrose could readily be induced, and that the change is a function of the humidity and temperature (within limits) to which the crystals are subjected. This observation forms the basis for a practical process of manufacture in which the anhydrous dextrose is stored in a closed chamber under controlled conditions of humidity and temperature until the desired change has been induced and for the process of producing a powdered flavoring compound by blending aqueous solutions of flavoring extracts with the quick hydrating sugar (59).

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CHAPTER XV

THE AMYLASES. PROPERTIES AND PRODUCTION

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1. Introduction and Historical. Enzymes may be defined as biocatalysts; that is, as organic agents found in living matter which have specific powers for accelerating or even initiating reactions without themselves being necessarily altered in consequence thereof. In modern nomenclature the name applied to a specific enzyme is designed in such a fashion that it will give some indication either of the substrate or the reaction for which the enzyme is specific. To this is appended the suffix "ase" to indicate an enzyme. Accordingly, enzymes which degrade starch or "amylum" are designated as amylases. This should be kept in mind and caution exercised that all enzyme reactions which result in the reduction of sugar polymers to simple sugars not be labeled amylolytic. By definition, if for no other reason, the term "amylolysis" should be reserved for those reactions in which starch is involved.

The amylase group of enzymes overshadows all others in industrial significance and utilization. This has led to extensive research since the discovery of "diastase" in about 1815. In the present treatment no attempt will be made to review extensively the almost innumerable publications. Such a review would constitute a sizable volume in itself. The reader will find a fairly adequate abstract bibliography of the literature previous to 1925 in Walton's text on starch chemistry (1). For the present purpose then it will suffice to give briefly the writer's concept of our present knowledge relating to the properties of those amylases of some industrial significance.

The industrial use of amylases appears to be as ancient as civilization itself. This use coincides in the beginning with the discovery that an intoxicating beverage resulted from the "fermentation" of cereal grains. It has been stated (2) that the earliest known records of the preparation of malt date back to 7000 B. C. and that beer brewing was an established craft by 5000 B. C. In the early history it may be assumed that knowledge of the mechanism of the starch-sugar transformation was very meager. The real foundation for the scientific approach to the problem of enzymatic starch degradation lies in the discovery of "diastase" by Kirchhoff (3) in about 1815 and the fine piece of research conducted by Payen and Persoz (4) in 1833. These latter authors named the active principle of barley malt "diastase," used the iodine-staining property of starch to follow its action, and even effected a degree of concentration and purification by alcohol precipitation.

Coinciding with the utilization of cereal amylases was an equally significant application of the amylases produced by the growth of microorganisms. It is evident that the alcoholic beverages produced in certain parts of the Orient

were and are in large measure dependent on the action of this type of amylase. Certain types of bacteria and molds, either as cultures or as contaminants, contributed starch-degrading as well as fermentative enzymes. Mold preparations of high amylolytic activity were used in Japan for centuries, but investigations of the nature of the action and the enzymes involved did not materialize until some time after the discovery and descriptions of cereal "diastase." Possibly the first study reported in English was that of Atkinson (5) in his treatise on the diastase of "Koji." His description of the action of this fungal preparation on starch and starch degradation products was rather complete. In addition he noted the now well known property of fungal amylases; *i.e.*, their sensitivity to heat.

The wide-spread use in the Occident of malt for diastasis stimulated much research on malt amylase, particularly that of barley malt. The "component" concept, the characterization of malt diastase as a mixture of α - and β -amylase, had its origin in the postulation by Mäcker (6) in 1879 that there are two "diastatic ferments" in malt. Although this postulate has since developed to be an accepted fact and has even progressed so far that many believe that all amylases from any source are mixtures of α - and β -amylase, this expansion of the component concept is entirely unwarranted and not supported by the available experimental evidence. The past half century has witnessed many investigations of amylases from a wide diversity of sources. While present knowledge is by no means complete, much is known of the mode of action and the properties of many of them.

Hand in hand with the development of a more complete knowledge of the amylases, a great expansion of their use has occurred. Far from their confinement to the saccharification of starchy mashes essentially for alcoholic fermentation is the present wide-spread use of the amylases in many diverse industries. Representative uses which may be cited are the preparation of sizings, desizing, preparation of special dextrins and sugars, and the clarification of sirups and other solutions containing undesirable starch and dextrins. These applications are treated adequately in another section of this book. It becomes apparent that amylolytic starch conversion is of pronounced industrial importance. It may be anticipated that this group of enzymes will assume ever more significance in the future.

2. Classification of Amylases. Several methods of classification suggest themselves. The enzymes are classified, for example, with respect to the substrates upon which they act. However, by definition, amylase action is typically confined to one type of substrate, starch. Differences exist in the manner in which native starch, starch pastes, and soluble starches are degraded, but fundamentally the action is the same, a cleavage of starch molecules. The action of amylase solutions in further hydrolyzing starch degradation products such as dextrins usually is considered as an amylase action. It is the writer's opinion that certain of such actions cannot truly be designated as entirely due to amylase.

Two logical schemes of classification are apparent. One of these would involve a division based on the type of starch degradation achieved, the other a division based on the source of the amylase. In the former, starch degradation is considered to be of three main types: liquefaction, dextrinization, and saccharification. It is questionable whether any one of these can occur to the exclusion of the others. However, one of the three may be the principal action, occurring in a much more striking manner than either of the others. This type of classification is the most useful for industrial purposes and when used in conjunction with other descriptive terminology is of great value. Corollary phrases may relate to such matters as thermostability, acid stability, pH optimum, etc. Assuming the availability of adequately described amylase preparations, a competent operator should have little difficulty in selecting the one which will most satisfactorily perform the function desired under any particular plant conditions.

The classification based on the source of the enzyme obviously is of more value to the amylase producer than to the user. Also, the characteristics of the amylases in any one group tend to be similar, and this can then be used as an aid in arriving at an enzyme product of desired properties. For example, if an amylase is desired that will liquefy starch at relatively high temperatures, greatest success would be anticipated by utilizing certain bacteria for production. The usual classification according to source consists of a division into three main groups: higher plants, microbes, and animals. The higher plants may be subdivided to indicate the particular plant, and again divided, especially with the cereals, to indicate whether or not the grain was germinated or malted. Amylases derived from the malts may be further divided into α - and β -amylase components. Thus, while "barley amylase" is a rather specific term, "barley malt amylase" indicates a mixture of amylases which have been resolved into barley malt α -amylase and barley malt β -amylase. Similarly, microbial amylases can be divided into groups, for example, fungal and bacterial amylases, and each division subdivided to indicate the specific organism used. Thus we have amylase from *Aspergillus oryzae*, from *Bacillus mesentericus*, etc.

It does not seem advisable to attempt a complete and detailed classification of the amylases. At best it would be cumbersome and present knowledge is not adequate to make it complete. It will suffice to keep in mind that three actions are important, starch liquefaction, dextrinization, and saccharification, and that the amylases of value in industry are derived from higher plants, particularly from cereal malts, from the growth of fungi and bacteria, and from certain glands of animals. The following sections of this chapter will outline the properties of the amylases, and the chapter by G. M. Severson will deal with the application or "use" phase.

3. Types of Activity and Evaluation.

A. General Considerations—The mode of action of an amylase essentially is that of an enzyme and more specifically of a hydrolytic enzyme. Since the kinetics of enzyme action have been discussed extensively (7-10), it is not neces-

sary to treat in detail the concepts evolved. Van Slyke (11) recently has presented an excellent treatise on the kinetics of hydrolytic enzymes and their relationship to the measurement of enzyme activity. In general the kinetics may be described as follows: in the initial stage of action, when the substrate concentration is great relative to that of the enzyme, the enzyme can act at full speed. The result is that under these conditions the rate of substrate degradation is proportional to the enzyme concentration and independent of that of the substrate; *i.e.*, the reaction curve is not "monomolecular" in character. In the later stages, when the substrate quantity becomes low relative to that of the enzyme, the pattern becomes that typical of monomolecular reactions. In the first stage there is a negligible time interval between combination of 1 molecule of substrate with the enzyme, its resulting hydrolysis and the release of the end-products, and the filling of the vacant space by another substrate molecule. In the second stage this time interval becomes appreciable, and the filling of vacant spaces on the enzyme is dependent on available substrate. This concept of the kinetics of hydrolytic enzymes fits some of the known facts regarding the actions of amylases on common starches (those staining blue with iodine). For example, in the saccharification of gelatinized starch by barley malt the amount of sugar produced in a given time is proportional to amylase concentration so long as the relative substrate concentration is high. However, after about 40% of the starch has been converted to sugar, the curve starts to break away from the linear relationship.

The above concept of enzyme kinetics is of prime significance in the measurement of amylase activity. As Van Slyke (11) points out, there are three possibilities for the designing of methods: (a) measurement of activity in the presence of an excess of substrate in order to observe conditions under which the reaction rate is proportional to the enzyme concentration, (b) measurement with substrate so dilute that the conversion simulates that of a monomolecular reaction, and (c) measurement based on the fact that, "with a given substrate solution, the time required to decompose a given fraction, say half, will vary inversely as the amount of enzyme present." All of these methods have been used in evaluating amylase activity. The various applications will be discussed under the specific types of action.

From the standpoint either of the producer or of the user, methods for evaluating amylase preparations are necessary adjuncts to successful practice. The principles for the present quantitative methods for evaluating the *saccharifying power* of malt were laid down by Kjeldahl (12) in 1879 with his "law of proportionality." He showed that up to about 40% starch conversion the rate of sugar production was proportional to the concentration of amylase. This discovery formed the basis for the later work of Lintner (13) whose "degrees Lintner" and "soluble starch according to Lintner" persist to this day. Somewhat earlier the disappearance of the iodine-staining property of starch was being used as a criterion for studying starch hydrolysis. This method for the evaluation of *dextrinization* was outlined as a quantitative procedure by Roberts (14) in 1881

and popularized by Wohlgemuth (15) nearly 30 yrs. later. The *liquefaction* of starch pastes by amylase preparations must have been one of the first actions observed. Through the years many methods for evaluating starch liquefying activity have been proposed but apparently few have the accuracy necessary for quantitative evaluation—a fact readily understood when the difficulties of preparing satisfactory substrates and devising adequate techniques are realized. Two of the more satisfactory current methods will be discussed later.

The methods developed for quantitative evaluation of amylases logically may be divided according to the three uses: liquefaction, dextrinization, and saccharification. Such methods for evaluating enzymes should not be confused with methods customarily used in the plant to test for the desired degree of starch degradation. These latter techniques have been developed primarily to measure a substrate change and not the exact activity of the enzyme present. They may take such forms as the approximate measurement of viscosity during liquefaction of the starch to a point previously found satisfactory for the purpose, or they may involve specific gravity measurements to determine whether or not saccharification has proceeded to a desirable degree. A difference between two amylase preparations may be noted but no attempt is made quantitatively to evaluate the preparations on an enzyme basis. Such methods are, however, of great value in plant practice. It is common knowledge that in certain processes the use, let us say, of twice as much enzyme does not result in twice as much conversion in the same time. It becomes necessary then to determine by practice either the optimum amount of enzyme to use under plant practice or, if a decrease in reaction time is desired, to determine the influence of enzyme concentration under actual working conditions. There are few data available which relate amylase concentration to the production of a desired commercial product, although such investigations appear to be highly desirable.

The following discussion of the specific reactions of amylases will be confined to the changes brought about by the action of these enzymes on gelatinized starch and for simplicity the action on starch giving a blue color with iodine. No attempt will be made to discuss the relative action on various starch "fractions" or the light which enzyme studies may throw on the structure of starch. Any specific methods for amylase evaluation that are described have been tested repeatedly by the writer and found satisfactory for the purpose.

B. Specific Actions of Amylases. Liquefaction—The decrease in the viscosity of starch pastes under the action of amylases has wide application in industry. The exact nature of the changes that take place during this liquefaction, however, is not entirely clear. It is well known, for example, that a viscous paste of gelatinized starch can be "liquefied" to a considerable degree simply by vigorous beating. The only apparent change during this process is a disruption of the swollen starch granules. Under the action of a liquefying amylase the change is very similar. During the first stages of liquefaction neither dextrinization nor the production of reducing groups can be detected except with difficulty. This has led some to believe that true enzymic starch liquefaction is nothing more

than a reduction of the starch aggregates to monomolecular size. Following this there would be, of course, a progressive splitting of the molecules resulting in dextrin formation and sugar production. During this secondary process a further pronounced reduction of viscosity would occur. On the other hand, the drop in viscosity could quite easily be attributed to the primary splitting of the starch molecules. This initial cleavage likewise would result in an inappreciable apparent dextrinization and in an insignificant production of reducing groups. Probably the most logical concept is that starch liquefaction consists of a reduction of starch aggregates to single molecules followed by a splitting of these molecules into dextrans of a high degree of complexity. It should be kept in mind, however, that this process is characterized by a very low production of reducing groups and an inappreciable change in the typical blue starch-iodine color of those starches originally staining blue with this reagent.

The kinetics of the liquefaction of starch by amylases apparently differ somewhat from those operative in dextrinization and saccharification. Over no range is there a linear relationship between the quantity of enzyme and the decrease in viscosity. This is not necessarily a failure of the general law relative to the early action of hydrolytic enzymes, but it is an indication that the reaction is of a different nature, and it may be that under experimental conditions the substrate-enzyme relationship necessary for this condition is not achieved. However, it does hold that with a given substrate and under limited conditions a doubling of the amylase quantity will cause a reduction to a desired viscosity in half the time.

Starch liquefaction typically is brought about by an amylase of the α type, and it may almost be taken for granted that, if an enzyme product is labeled α -amylase, it will have potent liquefying potentialities. As a matter of fact one of the most accurate methods suggested for measuring starch-liquefying ability is described as a method for the "determination of α -amylase" (16). Two methods have some current popularity in America for the quantitative measurement of starch-liquefying power. One of these was developed by Jozsa and Gore (17) and refined by Jozsa and Johnston (16). This method depends on the preparation of a standard starch substrate from potato starch and the use of a pipette viscosimeter. By careful gelatinization and beating, the viscosity is adjusted to that stipulated. Enzyme is then added and after a definite reaction time the viscosity is again measured. The results are related to a standard curve, and enzyme activity is calculated essentially at zero time of reaction. The unit values proposed for amylase content are designated "liquefons."

Another of the methods which has proved reliable is that proposed by Blom and Bak (18). In this method it is not necessary to adjust the viscosity of the substrate within as narrow limits as those stipulated by Jozsa and Gore (17). A pipette viscosimeter is also used but it is of smaller size and with a capillary inserted. Amylase activity is calculated from the time required to achieve a drop in viscosity from a stipulated point to another half as great. Both this method and that of Jozsa and Gore (17) have proved reliable in the hands of

experienced operators and appear to be satisfactory for the purpose of evaluating the potential starch-liquefying power of an amylase preparation under conditions conducive to enzyme stability.

Dextrinization—Most starches give a characteristic blue color when treated with a solution of iodine in potassium iodide. As enzymic splitting of starch occurs, carbohydrate molecules of less complexity result, and this is reflected in the color given with iodine. Dextrins of a low degree of complexity, approaching in characteristics the simple sugars, fail to give any color whatever when treated with iodine. This point has been called the achromic point. Between the two extremes of blue and colorless a range of colors dependent on dextrin complexity and presence or absence of residual starch is found. These colors visually range through the purples, the reds, and the browns. It may be seen that dextrinization is a complex process, since the end-products are of considerable variability. For the present purpose, dextrinization will be considered as the production from starch of sugar polymers of lesser complexity than starch. These polymers characteristically have a low viscosity relative to that of starch and a low reducing power relative to that of maltose or glucose.

When an amylase of the α type is allowed to act on gelatinized starch, the characteristic drop in viscosity described above occurs. Further action results in a progressive conversion of the substrate to products which stain purple with iodine, then red, and finally give no color whatever. Characteristically, with amylases of this type a low production of reducing groups accompanies this change. Another type of dextrinization is that characteristic of the β type of amylase (*e.g.*, the amylase of ungerminated barley). In this instance the starch is rapidly converted in large degree to maltose and a " β -amylase limit dextrin." The combined end-products stain purple with iodine. If the β -amylase used is uncontaminated by α -amylase, the reaction apparently ceases at this point, and no further degradation of the dextrin occurs. (This dextrin frequently has been designated α -amylo-dextrin.) This particular form of starch degradation may be termed a partial dextrinization and a partial saccharification, since maltose production is rapid but not complete and the dextrin produced is complex. A third type of enzymic starch dextrinization is that resulting from the action of a combination of α - and β -amylase (*e.g.*, barley malt). In this case a rapid splitting of the starch to products which fail to give a blue color with iodine is coincident with an equally rapid production of reducing sugars.

An unusual type of enzymic dextrin formation is the production of non-reducing crystalline (Schardinger) dextrins. These dextrins characteristically result from the action of the amylase produced by cultures of *Bacillus macerans*. Recent studies (19, 20) on the action of this amylase indicate that, following a sharp drop in viscosity, there is a gradual production of dextrins of the Schardinger type. These dextrins characteristically have low but appreciable reducing power. Kerr (21, 22) has postulated that such dextrins are not true degradation products or "limit dextrins" but the result of a synthesis from

simpler configurations. The production of "Schardinger dextrins" appears to have little if any industrial significance at the present time.

It is apparent from the above that great care must be exercised in the selection of an amylase to bring about the production of a certain desired type of dextrin. However, the reduction of starch to products which are still complex but which fail to give a blue or purple color with iodine is characteristic of all amylases except pure β -amylase. Accordingly, this technique has been the basis of one type of method for evaluating amylase activity. Obviously such technique is not directly applicable to investigations of the dextrinization of starches staining red with iodine. These "waxy" starches are receiving intensive investigation at the present time. Available data are not sufficient to define clearly the nature of their amylolytic degradation.

Some of the first methods for evaluating dextrinization were based on the hydrolysis of starch to products which fail to give any color with iodine. This achromatic point is difficult to detect, and the popularity of the method of Wohlgemuth (15) may have been due in large part to his selection of a pinkish red end-point. In essentials his method consists of the addition of varied amounts of enzyme to a standard starch substrate and appraisal of the color given with iodine after a given time under standard conditions. Quantitative evaluation is based on the amount of enzyme necessary to bring about the desired change under the standard conditions. This technique has had considerable modification. A recent modification (23) which has gained some degree of popularity in malt evaluation is based on the time necessary for an enzyme to convert starch to products which give a red-brown coloration with iodine. The standard end-point is the color given by a specified combination of dextrin and iodine. The dextrinization time is inversely proportional to the concentration of amylase, and "dextrinogenic units" (24) are calculated as the number of grams of soluble starch dextrinized by 1 g. of malt in 1 hr. at 30° C. A further advantage of selecting as an end-point the red or red-brown color given with iodine is that the dextrinization measured by its use is proportional to the quantity of enzyme.

For many years, such dextrinization techniques were considered to be specific measures of α -amylase and unaffected by β -amylase. Blom, Bak, and Braae (25), Hanes and Cattle (26), and Sandstedt, Kneen, and Blish (23) independently showed that by the procedure the combined action of the two amylase components of malt is measured and it is therefore partially dependent on β -amylase activity. The latter of these workers (23) proposed a dextrinization method specific for the α -amylase present in malt; *i.e.*, the measurement of the dextrinization rate in the presence of an added excess of β -amylase. This method has proved adequate for accurate evaluation of the α -amylase activity of cereals, but its use with other amylases has not been investigated. In any case, the use of an excess of β -amylase in studying the dextrinizing activity of the many preparations which contain no β -amylase would seem unwarranted.

Other methods for the evaluation of the dextrinizing or "amylolytic" activity of amylases have been suggested; *e.g.*, the alcohol-precipitation method

of Caldwell and Hildebrand (27). However, evaluation by iodine-staining properties appears to be the most accurate, the most adaptable to laboratory conditions, and therefore the most popular.

Saccharification—Starch in its unaltered form has a negligible reducing action on such oxidizing agents as iodine, potassium ferricyanide, and alkaline copper sulfate solutions. The action of amylases, and particularly certain amylases, on this substrate causes a production of compounds which have marked reducing properties. When this splitting of starch results in the production of sugars, the process involved is termed saccharification. In addition to their reducing properties, these sugars are fermentable by yeast, and this in turn forms the basis for the very extensive use of amylases in the saccharification of starchy mashes for subsequent alcoholic fermentation. The importance of this type of conversion has stimulated much research on the nature of the process.

It is generally agreed that the principal sugar resulting from the enzymic saccharification of starch is maltose. Glucose has been found in the products, but there is some question as to whether this is the result of amylase action or of the further splitting of maltose and other glucose polymers by enzymes other than amylase. At any rate, the common procedure in evaluating starch saccharification is to calculate the sugars produced as maltose and append the phrase "calculated as maltose" to indicate uncertainty as to the exact nature of the products. This difficulty can be circumvented in some degree by yeast fermentation of the products and measurement either of the alcohol or the carbon dioxide production.

It is usually the case in industrial saccharification that a high degree of conversion of starch to sugar is desired. In such a conversion the kinetics of the reaction are of the two-phase nature described earlier in this chapter. In the first of these, occurring when the ratio of substrate to enzyme is high, sugar production is rapid and linearly related to enzyme concentration. The second phase is a much slower reaction and not linearly related to enzyme concentration. The foregoing holds for β -amylase action and for the action of enzyme preparations containing both α - and β -amylase. In the case of hydrolyses in which the only amylase operative is the α form, conformity to the first phase of saccharification is difficult to detect. In no range is there a linear relationship between production of reducing groups and amylase concentration. This is not surprising when it is considered that there is a complete disappearance of whole starch from the reaction medium before appreciable sugar production can be detected, the majority of saccharification being due not to a direct action on starch but to a further splitting of dextrins.

In addition to the two phases of the saccharification process, there are at least three main types of starch saccharification by amylase. One of these is that well known from observations of barley malt. A rapid dextrinization occurs accompanied and followed by a rapid production of maltose. In the later stages, the reaction slows down, but sugar production proceeds steadily until the extent of conversion approaches complete degradation of the starch to sugar.

This reaction is typical of the combined actions of α - and β -amylase. A second main type of saccharification is that performed by the amylase typical of ungerminated barley, β -amylase. Saccharification is rapid and linearly related to amylase concentration in the first phase. Sugar production slows down in the later stages and terminates when some 60% of the starch has been converted to maltose (with pure β -amylase and at least several of the starches this termination coincides almost exactly with the 60% level). If the β -amylase is uncontaminated with α -amylase, this limit dextrin, at least in so far as its iodine-staining properties are concerned, remains apparently unchanged for months.

A third type of saccharification is that typical of preparations designated as α -amylases. Since most amylases appear to be of this type, the reaction is important. These amylases are thought of as starch-liquefying and dextrinizing enzymes and justly so, since their early action predominantly is of that nature. However, small amounts of fermentable sugar in addition to reducing dextrins are produced during the dextrinization process, and following dextrinization a steady production of sugar occurs. This saccharification, if the enzyme is not inactivated on standing and if sufficient time is permitted, frequently attains as high a level of starch conversion as that characteristic of the action of barley malt. Rapid conversion to sugar may be achieved if relatively massive quantities of the enzyme are used. Certain α -amylase preparations appear to be deficient in this postdextrinization capacity for saccharification, and those that have the facility vary greatly in this regard. This evidence coupled with the usual appearance of glucose in the end-products of α -amylase action suggests that other enzymes may be operative in these later stages of saccharification. Kerr and coworkers (28, 29) have demonstrated that such an enzyme, α -glucosidase, has a rôle in the conversion of certain limit dextrins. Unpublished data of the writer strongly indicate that such an enzyme is present in certain cereal malts as well as in certain fungal and bacterial preparations and may greatly influence the production of sugars by α -amylase preparations.

Methods for the evaluation of the saccharifying power of an amylase preparation in general have had one characteristic in common: they have been designed so that Kjeldahl's law of proportionality (12) is followed. In other words substrate concentration, time, and quantity of enzyme are adjusted so that starch conversion does not exceed 40%. They are then the same in principle. Standard conditions relative to time, temperature, pH, and substrate concentration are followed, and the sugar production is determined either by the reducing properties of the products or by other means such as optical properties or fermentability. If saccharification is calculated in terms of sugar production, the products formed usually are grouped and calculated as maltose. So long as the appropriate conversion limit is observed, the unit values used to designate enzyme activity are equally reliable. For example, when the regular Lintner method and the rapid 15 minute method of Kneen and Sandstedt (30) are used to determine the activities of the same series of malts, the relationship between "degrees Lintner" and "Kneen-Sandstedt units" is a linear function, and either "unit"

may be arrived at by applying a factor to the other. It is apparent then that the method used will depend on custom and convenience. The limitations of prevalent methods for measuring saccharifying activity should be kept in mind. By those such as the Lintner or the Kneen and Sandstedt (30) technique the combined saccharifying activity of all the amylases present is measured and this activity is measured only in the early stages of starch saccharification. It is only recently that an accurate evaluation of the amount of saccharification attributable to the α and β components of malt has been possible. The development of a method (30) for the determination of β -amylase activity in the presence of α -amylase and its use with barley malts (24, 30) have shown that *on the average* 85% of the saccharifying activity of a barley malt is due to β -amylase and that the other 15% is due to the α component. Further, in the procedure of a manufacturing plant a type of saccharification extending over several days may be practiced. In this instance, *e.g.* the production of alcohol from starchy mashes, the saccharification need not be the very rapid conversion characteristic of the action of barley malt on gelatinized starch but only one supplying fermentable sugar in amounts adequate for yeast capacity. For such processes the "Lintner value" of an enzyme product may be of little value; the utility of the product may depend on the content of α -amylase and other enzymes not adequately measured by a method largely dependent on β -amylase activity.

The evaluation of amylolytic activity whether it be based on saccharification, dextrinization, or liquefaction is a necessary adjunct to standardization of preparations and to the division of such preparations into the various utility groups. Such evaluations serve, of course, only as guides in the selection of an enzyme for industrial use and the prediction of its value therein. It should be emphasized again that until adequate correlations are worked out between known amylase concentrations and properties and the manner in which these are operative under plant conditions, final evaluation can be made only by trial.

4. Properties of Amylases.

A. General Properties—It is not within the scope of the present treatment to deal extensively with the development of the present views regarding the nature and the kinetics of action of amylases. Present evidence indicates strongly that amylases either are protein entities or are specific groupings intimately associated with specific proteins. From Kirchhoff's identification of the characteristic action as associated with the "gluten" of cereal grains, through Osborne's researches (31, 32) and the multitude of papers following, the properties of this enzyme have appeared as those typical of proteins. All amylases are irreversibly inactivated by high concentrations of acid or alkali, and by boiling temperatures. They do not pass through cellophane dialysis membranes. They are soluble in water or very dilute salt solutions and in dilute ethanol solutions. At higher levels of salt (ammonium sulfate) and higher concentrations of ethanol they are precipitated in forms that are active when redissolved in water.

The activity of an amylase is dependent on a number of factors. The rate of reaction may or may not be influenced by various salts, protein cleavage

products, lipids, etc., and by other substances which have been classed as specific activators and inhibitors. It is becoming increasingly apparent that most of the so called activators function either to increase the solubility of the amylase or to increase its stability. For instance, many regard the calcium ion to be an "accelerator" of reactions based on malt α -amylase activity. Actually, calcium serves no other purpose than to increase the stability, *i.e.* decrease the loss of activity, of this enzyme. As a further instance of the complexity of these stability factors it has been shown (33) that the presence of calcium ions decreases the stability of β -amylase, the other amylase component of malt. In neither case does calcium have any influence on the early rate of reaction but merely influences the rate of loss of enzyme activity.

It is commonly accepted that the end-products of amylase action have an inhibitory effect on the further action of the enzyme. Other inhibitory substances of a more specific nature have been described. Examples are provided by the insoluble substance present in germinated buckwheat (34, 35) and the water-soluble, protein-like substance (36) present in wheat, rye, and certain of the sorghums. The latter appear to be true inhibitors inasmuch as they are specific for certain amylases and have no influence on others. The actual inhibition has the appearance of an adsorption and may be reversed to liberate the amylase in its original active form.

From an industrial view-point, perhaps the most important characteristics of amylases, aside from the type of conversion they catalyze, are those relating to the influences of *pH* and temperature. All amylases have *pH* optima for their actions. The *pH* optimum for any specific amylase will depend on the duration and temperature of the reaction. This apparent anomaly results from the difference between the *pH* optima for activity and for stability. For example, malt α -amylase is most stable at a *pH* close to 7.0, but the optimum for activity at 30° C. is about *pH* 5.0. If the reaction temperature is raised sufficiently to cause a progressive inactivation of the enzyme, the apparent optimum *pH* will be raised accordingly. In other words the apparent optimum activity is a compromise between the actual optimum and that at which the amylase has the greatest stability. Similarly, under conditions contributing to instability, the longer the reaction time, the closer to neutrality the *pH* optimum for activity becomes. In industrial utilization therefore it is important that an amylolytic conversion be carried out at a *pH* which is optimum under the particular conditions operative and not necessarily at a *pH* which may be optimum under any other set of conditions.

The relationship between amylase activity and reaction temperature likewise is of utmost significance in industrial application. In low temperature ranges, for example between 20° and 30° C., the rate of conversion is approximately doubled by a rise in temperature of 10° C. As the temperature is raised to higher levels, the increase in reaction rate with increase in temperature diminishes until a point is reached at which further increase in temperature has no apparent influence. In this region the rate of irreversible loss of amylase exactly balances

the increase in activity of the remaining amylase. Further increase in temperature causes a more pronounced loss of enzyme and, eventually, complete destruction of the amylolytic power of the preparation. Since loss of enzyme activity is a function of time and pH as well as of temperature, it is obvious that these factors likewise must be taken into account. An optimum temperature for amylase activity does not exist, merely a temperature which, under the particular working conditions, will cause the most rapid completion of the change desired.

Amylases from different sources have different degrees of thermostability. The rise in popularity of bacterial preparations may be attributed almost entirely to the thermostable nature of some of these amylases in the combined gelatinization and degradation of certain starches with high temperatures of gelatinization. Under other conditions when resistance to thermal inactivation is not as critical a factor, an amylase deficient in this respect can be utilized. For any amylase of the α type, *i.e.* primarily a starch-liquefying and dextrinizing enzyme, the resistance to thermal inactivation can be increased by certain adjustments in working conditions. The more important of these are the adjustment of the pH to one more nearly approaching the optimum for stability, and the addition of calcium ions. It thus becomes apparent that an amylase preparation of low thermostability in the natural state may be made to approach in this regard one of higher stability. Two types of stability are apparent: the inherent stability of an amylase, and the stability conferred upon the enzyme by substances which either occur naturally in conjunction with the amylase or are supplied as adjuncts.

B. Specific Properties—As discussed above, it becomes somewhat difficult to state the properties of a specific amylase without elucidating exactly the conditions under which such properties are measured. Accordingly, the properties of the various amylases will be described in rather general terms but with the intent of differentiating clearly between those from different sources. Very few adequate comparative studies have been reported, and some of the specific data available in the literature are contradictory. The conclusions expressed are those arrived at by examination of much of the literature and from data, some of which are unpublished, obtained by the writer and collaborators. The discussion will be divided into considerations of the three principal groups of amylases, those from higher plants, those of microbial origin, and those from animals. No attempt will be made to deal with all the amylases; attention will be directed to those which, at the present time, have potential industrial significance.

1. Amylases of Higher Plants—Amylases of this group were perhaps the first used industrially. Their properties will receive more attention in the discussion than may seem warranted but there are several reasons for this. Extensive research has been carried out on this group, particularly on the amylases of barley malt; hence there is accurate and abundant information. Barley malt, among others, contains the two amylase components, α - and β -amylase, and therefore lends itself particularly well to a discussion of these components. Since

all amylase properties are of relative degree, moreover, it is convenient to use the barley amylases as standards for comparison.

For the present purpose, the higher plants may be divided into two groups, one dealing with the cereals and the other embracing plants outside this group which may have industrial significance. The cereals may be divided into two subgroups, the malts and the ungerminated grains.

Cereal Malts—The best known member of this group is barley malt. Since its amylase system is composed of two components, α -amylase and β -amylase, the starch-degrading properties of barley malt are those of the combination of these components found in the particular sample. One malt may be high in β -amylase and low in α -amylase, another just the reverse. Of the three main functions of amylases only one (24), starch liquefaction, appears to be influenced by a single component, α -amylase. Dextrinization is greatly influenced by both components, and, while saccharification as customarily measured is largely dependent on β -amylase activity, a minor rôle is played by the α component. The properties of these two amylase components vary widely and will be discussed separately. Varying nomenclature has been applied. Wijsman (37) in 1890 made a clear separation of the two and called them "dextrinase" and "maltase" to describe their dextrin-producing and maltose-producing properties. (The "maltase" referred to in the older literature as a diastatic agent should not be confused with the maltose-splitting enzyme.) Kuhn (38) in 1925 proposed the nomenclature of " α -amylase" and " β -amylase" and indicated that they produced respectively α - and β -maltose. At about the same time Ohlsson (39, 40) proposed that they be called "dextrinogenic amylase" and "saccharogenic amylase." At present the terms α - and β -amylase are the most popular, though customarily they are not used in the sense implied by Kuhn. Rather, α -amylase is thought of as the starch-liquefying and starch-dextrinizing enzyme, β -amylase as the typical starch-saccharifying enzyme. Characteristically α -amylase is the amylase of "activity," β -amylase the amylase of "dormancy." In general it appears that if amylase is synthesized as a result of active growth, the α form predominates; if laid down in conjunction with starch in reserve or storage organs the β form predominates.

Barley Malt α -Amylase—This amylase acts on "raw," native starch (24), and readily liquefies and dextrinizes gelatinized starch with a minimal early production of sugar. Essentially the action is a breaking of the molecule into large fragments (41). There is a slow production of sugars during the later stages of its action on gelatinized starch, but eventually a high degree of saccharification may be attained. The pH optimum for activity at room temperature is in the neighborhood of 5.0. The amylase is relatively unstable, especially in dilute solutions, and its stability is remarkably enhanced by the presence of calcium ions. Inactivation is more rapid at a low than at a high pH and the pH optimum for stability is approximately 7.0. It is resistant to surface denaturation and has a fair degree of thermostability. At pH 6 to 7 and in the presence of calcium, loss in activity at 70° C. is slow. α -Amylase is soluble in

water or the very dilute salt solutions resulting from extraction and is rendered more soluble by the peptizing action of either dilute salt solutions or the proteolytic enzyme papain. More concentrated salt solutions (e.g., 25 to 35% ammonium sulfate) cause precipitation. α -Amylase is readily soluble in dilute ethanol solutions but precipitates at levels in the neighborhood of 60% ethanol.

Barley Malt β -Amylase—This amylase does not act on most native starch granules and liquefies and dextrinizes gelatinized starch inefficiently. Saccharification of gelatinized starch is rapid but incomplete, ceasing with many starches when conversion to about 60% maltose and 40% "limit dextrin" has occurred. Saccharification is achieved by the breaking away of single maltose molecules from the ends of chains or of chain branches (41). The pH optimum for activity, near room temperatures, is about 4.5. This component is relatively stable at room temperature, especially in the absence of calcium ions. Inactivation is much more rapid at high than at low pH values and the pH optimum for stability appears to be in the neighborhood of 5.0. It is sensitive to surface denaturation and has a low degree of thermostability. Thermal inactivation is largely completed after a few minutes at 70° C. Solubility characteristics are similar to those given above for α -amylase except that higher concentrations of ammonium sulfate or of ethanol are required for complete precipitation and dilute salt solutions or papain-like enzymes effect a greater release into solution.

The above description of the components of barley malt amylase indicates the reasons for the versatility of this product. Malts of the other cereals may be divided into two groups. The first group is similar in amylase composition to barley malt.

Malts of Wheat and Rye—These malts have both α - and β -amylase as components of their starch-degrading systems and in somewhat similar amounts and relative proportions. The respective components of these malts are similar in their essential properties to the α - and β -amylases of barley malt. Minor differences do occur but may be due to a variation in substances accompanying the amylase in extracts rather than to a fundamental difference in the enzyme. For example, barley α -amylase appears to have greater thermostability than wheat α -amylase, whereas the relative thermostabilities of the β components are just reversed (33). As a consequence of the similarity in amylase composition, the malts of barley, wheat, and rye, exhibit similar actions in respect to starch liquefaction, dextrinization, and saccharification.

Malts of Oats, Sorghum, Maize, and Rice—The three cereal malts discussed above have in common the presence, in addition to α -amylase, of considerable quantities of β -amylase. Those now treated may develop similar levels of α -amylase on germination, but all are characterized by a deficiency of β -amylase (42). In fact, this component is present in such small amounts that its activity usually does not become apparent when macro-methods of evaluation (13, 30) are employed; however, the use of micro-techniques permits an estimation of the levels present (42).

The α - and β -amylases of oats, sorghum, maize, and rice appear to be quite similar in properties to the corresponding amylases of barley malt with, of course, minor variations. The pH optima for activity, thermal reactions, and solubilities are comparable. The mode of starch degradation of the whole malts is similar to barley malt α -amylase in the early stages, up to the point of complete conversion of the starch to low molecular dextrans. Following dextrinization, sugar production proceeds at a rate faster than that characteristic of α -amylase to a degree governed by the level of β -amylase present. Since wide varietal differences may occur, both in α -amylase activity and in the micro-quantities of β -amylase present (unpublished data), the rate of this postdextrinization saccharification is a characteristic of the particular malt used.

It has been well known that the malts of such cereals as sorghum and maize are low in the type of saccharifying action typical of β -amylase activity. Nevertheless, sorghum malt has been used with success in India (43) and in South Africa (2) for the conversion of starch to fermentable sugar. It appears that the presence of a large proportion of β -amylase in a malt (high Lintner value), while frequently desirable, is not an essential feature. Further, the wide variations in the amyolytic properties of malts emphasize that the common term "malt," as used to characterize a certain type of amylase, is a misnomer.

It is appreciated that the starch-degrading properties of malts are not the only characteristics of significance in industry. By far the greatest percentage of malt is employed in the production of alcoholic beverages. In these instances, such qualities as flavor receive necessary consideration. However, if the cereals are evaluated solely on the basis of amylase development during malting, certain ones have more value than others. Among the "cool climate" cereals, barley malts appear to average somewhat higher in β -amylase activity than malts of the others. Oat malt is very low in this respect, and the malts of wheat and rye lie close to that of barley. Wheat appears to have superior ability for the synthesis of α -amylase during germination and again oats are low. Of the "warm climate" cereals, certain varieties of sorghum, particularly "kafr," appear to be superior in ability to synthesize α -amylase, and the malts may achieve high levels of this component. Selection of a malt to perform a definite type of starch degradation should be based on the content of the desired amylase unless other properties preclude its use.

Ungerminated Cereals—Ungerminated cereals invariably have lower amyolytic powers than the malts produced by germination. As with the malts, they may be divided into two groups, based on the content of β -amylase. Most appear to have microquantities of α -amylase present even in the well ripened grain (42). The cereals that produce malts having high levels of β -amylase activity are relatively well supplied with β -amylase in the ungerminated state; those having a deficiency of β -amylase activity when malted show very low activities before germination. Accordingly, barley, wheat, and rye contain an active β -amylase in considerable quantities, and the starch-degrading properties of their extracts are those characteristic of β -amylase. In addition, traces of

α -amylase are present. A large proportion of the amylase contained in the grains is difficultly soluble, and extraction is markedly increased by the use of dilute salt solutions or papain-like proteolytic enzymes. However, even if the amount of α -amylase extracted is doubled or tripled by these procedures, it still is relatively insignificant and contributes little to the typical β -amylase degradation of starch performed by such extracts.

Extracts of the ungerminated grains of sorghum, maize, and rice are practically inactive from an amylase standpoint. Though ungerminated oats have appreciable β -amylase activity, it is limited to about one-tenth of that found in barley or wheat (42). All show the very low, but measurable, liquefying and dextrinizing ability characteristic of the traces of α -amylase usually present; when compared to the α -amylase activities of the malts this is of negligible degree.

Higher Plants Other Than Cereals—It is evident that practically all plants, as well as animals, utilize amylase activity in their physiological processes. However, in only certain instances is the concentration of these enzymes sufficient to offer industrial possibilities. With the higher plants such concentration, if present, occurs in the storage organs. Two sources of amylase have been suggested: the seed of soybeans and the underground storage organs of the sweet potato plant.

Newton and coworkers (44, 45) have conducted investigations of the properties of soybean amylase. They characterize the enzyme as β -amylase inasmuch as it has starch-saccharifying powers similar to those exhibited by β -amylase from cereals. Their data indicate that the amylase is present in considerable quantities in the ungerminated bean and can be extracted with water and concentrated by precipitation with 65% ethanol. The suggestion is made that soybean β -amylase has a higher degree of thermostability than cereal β -amylase. In unpublished investigations the writer has found that soybean extracts contain small amounts of an α type of amylase in addition to the typically saccharogenic amylase. This indicates that, as with the cereals, a procedure for differentially inactivating the traces of α -amylase must be employed before the amylase may be used in studies necessitating "pure" β -amylase.

The fact that the sweet potato is rich in diastase was noted by Gore (46) over 20 yrs. ago. Giri (47, 48) made extensive studies of this amylase and found it to be very similar to barley malt β -amylase in its action on starch and in its properties. The action of this amylase has some value in the preparation of sweet potato sirups.

2. *Microbial Amylases*—The discussion of these will be divided into considerations of the properties of amylases produced by the growth of the fungi and of the bacteria.

Fungal Amylase—The fungal amylase most widely produced and therefore best known is that resulting from the growth of appropriate strains of *Aspergillus oryzae*. This amylase is very similar in starch-degrading properties to malt α -amylase with the exception that postdextrinization saccharification is more rapid. The characteristic high liquefying power is present, and dextrinization is

rapid and coincident with a low production of sugars. The later stages of the action on gelatinized starch are marked by a steady production of fermentable sugars. The fact that this saccharification proceeds to high levels of starch conversion and is sufficiently rapid to meet yeast requirements is evidenced by the efficiency of some fungal amylase preparations in alcohol production. The pH optimum for activity is about the same as that of malt α -amylase; namely, 5.0. Calcium acts as a stabilizing factor. Solubilities in salt solutions and ethanol are similar to those of the other amylases. Fungal amylases characteristically are less resistant to thermal inactivation than malt α -amylase, and therefore their apparent temperature optima for activity are lower. At 70° C. the loss in activity is very rapid.

Bacterial Amylases—The commercially available amylase preparations resulting from the growth of bacteria appear to be of the α type, with starch-degrading properties similar to those of malt α -amylase (25, 49). Unpublished data obtained in the writers' laboratories support this conclusion but, in addition, confirm the finding of Effront (50) that it is possible to produce bacterial preparations with much greater saccharifying potentials than that typical of α -amylase.

The bacterial amylase available for industrial use typically is a starch-liquefying and dextrinizing enzyme with low saccharifying power. Like other α -amylases, its activity is stabilized by calcium ions. Solubility characteristics are similar to those of the other amylases. The pH optimum for activity is somewhat higher than that for malt α -amylase, being close to pH 7.0. The bacterial amylases have a greater degree of thermostability than any other of the commonly used amylases and may be used with little loss of activity at temperatures above 70° C. This thermostability, resulting in a high apparent temperature optimum for activity, is the most important attribute of this class of amylases and is the chief factor contributing to their expanding use.

There is no need to discuss further the amylase resulting from the growth of certain strains of *Bacillus macerans*, an amylase that functions as an α -amylase only up to the point of complete dextrinization of starch. The production of "Schardinger dextrans" rather than further degradation to sugars appears to be typical of this specific amylase preparation. The existence of such an amylase does indicate, however, that no matter how definite the statements above may appear they apply only to products which have engaged the attention of workers in the field. New preparations of bacterial "amylases," or of fungal "amylases," may have entirely different properties not only with regard to the potentialities of the amylases but with respect to other carbohydrate-splitting enzymes which may modify the saccharification reaction.

3. *Animal Amylases*—These embrace a multiplicity of amylases: pancreatic, salivary, and the amylases of urine, blood, etc. The amylase secreted in the pancreas appears to be the only one of any present industrial significance, and the discussion will be limited to it. The other animal amylases are similar in starch-degrading action and in properties to that derived from the pancreas.

Pancreatic amylase preparations are typical α -amylases with the usual starch-degrading properties characteristic of this enzyme. There is no evidence that β -amylase or an amylase with β characteristics is present. Accordingly, the preparations essentially are starch-liquefying and starch-dextrinizing in nature. Relative to these actions the saccharifying potential is low, with most of the sugar production occurring after dextrinization has been completed.

In common with all animal amylases, pancreatic amylase is very sensitive to neutral salts and requires their presence in solution for maximum expression of amylase activity. Dilute solutions (0.1 to 0.5%) of sodium or calcium chloride are commonly used, and it appears that such salts can truly be called "accelerators" and not just "stability factors." The nature of this acceleration is obscure. It seems probable that the colloidal suspensions of these animal amylases differ from those of other amylases perhaps in degree of protein dispersion. It may be postulated that the neutral salts act as peptizing agents in a manner to increase the solubility and activity of the amylase protein. In addition to their sensitivity to salt, animal amylases are distinguished by having pH optima for activity at somewhat higher ranges than those for the majority of the plant amylases. Under ordinary conditions pancreatic amylase shows its maximum activity at about pH 7.0.

Pancreatic amylase is of low thermostability, in this respect being at a level below malt α -amylase and at one similar to fungal amylase. As in the case of other α -amylases, calcium acts as a stabilizing factor. Other properties such as solubility in dilute salt and ethanol solutions and precipitation at higher levels of such reagents appear to be similar to those of the amylases in general.

5. Production of Amylases. The chapter would not be complete without a brief discussion of the manner in which preparations of desirable amylolytic properties are obtained. In some instances, as with the storage organs of plants and certain glands of animals, the amylase need only be extracted. In other cases a synthesis of amylase is brought about; *e.g.*, in the germination of cereals and in the growth of fungi and bacteria.

A. Production by Higher Plants—As a plant approaches maturity, considerable concentrations of complex carbohydrate and of protein may be deposited in storage organs. During the progress of the deposition, an amylase may be laid down in close association with the starch and other proteins. This "amylase of storage" typically is a β -amylase. As pointed out in the preceding section, this deposition of β -amylase is not universal and may be the exception rather than the rule. The seeds of certain cereals have it in large quantities, others in traces or not at all; most legume seeds are deficient in amylase but soybeans have liberal supplies. Its presence in abundance is not evident in all underground storage organs but certain ones such as those of the sweet potato are excellent sources.

β -Amylase relatively free from the α form does not have a great deal of industrial importance. If such an amylase is desired, it may be extracted from appropriate sources such as barley, wheat, rye, soybeans, and sweet potatoes.

Extraction is readily accomplished by the use of dilute neutral salt solution such as 1% sodium chloride. Papain may be a valuable adjunct to extraction, especially in the case of the cereals. Once obtained in solution the β -amylase may be used "as is" or concentrated and partially purified by precipitation by neutral salts in high concentration or by 80% ethanol. The precipitate is separated, dried, and handled as a stable powder. Details of a method for preparing β -amylase free from the α form are given by Kneen, Sandstedt, and Hollenbeck (33).

When the seeds of the cereals are placed under favorable growing conditions and allowed to germinate, a rapid production of α -amylase almost invariably takes place. For those grains originally containing β -amylase there is a concurrent increase in solubility of this stored amylase caused, it is believed, by the action of proteolytic enzymes simultaneously developing. Conditions adopted for germination or "malting" of the grains are selected to suit the particular cereal. For example, the low temperatures customarily used in the malting of barley are entirely unsatisfactory for the germination of sorghum. This cereal and others of like nature require considerably higher temperatures to promote active growth.

The malting process from the grain to the finished malt includes in its essentials the selection of a desirable grain which is cleaned, steeped, or soaked to an appropriate moisture content, and then allowed to germinate either on a "floor" or in "drums" with appropriate moisture and temperature conditions and with adequate aeration, and finally dried. The process will vary, depending on whether a "brewers' malt" or a "distillers' malt" is the desired product. For example, in the production of a distillers' malt conditions favorable to the development of high amylase activity should be observed, and drying may be carried out at the relatively low temperatures necessary for the preservation of this activity. On the other hand the yield and nature of the extract are of greatest significance in the production of brewers' malt, and no attempt is made to develop and maintain an amylase activity higher than "adequate." For a complete treatment of the biochemistry of malting a standard reference such as Hopkins and Krause (51) should be consulted.

In the preceding section of this chapter it was indicated that malts vary widely in their amylolytic activities. Detailed information relative to the influence of the kind of cereal, the variety, and the growth environment on the malting quality of cereals cannot be included in this discussion. It must suffice to say that a malt of almost any desired amylase content and ratio of amylase components can be prepared by proper selection of the cereal and of the production conditions.

Depending on the purpose for which it is to be employed, the dry malt may be either ground and used as such, milled to produce a malt flour, or extracted. Water extracts may be used or concentrated at low temperatures to give diastatic sirups. In such extracts or sirups the α -amylase is relatively unstable, particularly when a deficiency of calcium exists. Accordingly, storage and handling of

concentrated malt amylase preparations are best accomplished in the form of the precipitates obtained by neutral salt or alcohol precipitation.

B. Production of Microbial Amylases—Individual fungi and bacteria vary greatly in their potentialities for producing amylases and excreting them into the media on which they are grown. Not only do certain groups appear to have greater potentialities than others, but strains within a group vary markedly in this property. The only distinguishable difference between two such strains may be in this property. In the selection of an organism to be used in production two possibilities are apparent: A strain previously shown to be valuable may be employed, or a new strain may be isolated. If the latter method is chosen, it becomes a matter of collecting all available strains of a group known to have promise, of comparing their amylase production, and of selecting the most promising individual strains. The dependency of amylase production by any one strain on the medium employed and on the growth conditions further adds to the complexity of the process.

Fungal Amylases—These amylases customarily are produced by the growth of selected fungi on solid or semisolid media. Only the general features of production will be given here. More detailed information may be obtained by consulting the papers of Takamine (52, 53), Oshima and Church (54), Harada (55), and Underkofler and coworkers (56).

Various types of media have been used for growing the fungi. The most popular material at present appears to be wheat bran. Various fungi have been used, but strains of *Aspergillus oryzae* have proved most popular. Hao and coworkers (57) prepared "mold brans" by the growth of members of the genera *Aspergillus*, *Mucor*, *Penicillium*, and *Rhizopus* on wheat bran and concluded that the better strains of *Aspergillus oryzae* were most satisfactory.

In general terms, the preparation of "mold bran" may consist of cooking a mixture of equal parts of wheat bran and water, cooling, inoculating with the fungus, maintaining at 30–35° C. with adequate aeration for several days, and finally drying at low temperatures. The whole process, including culture of the mold, preparation of the inoculum, and subsequent growth must be performed in such a manner as to minimize the possibility of contamination. Dilute acid may be added to the medium before growth; adjustment to about pH 4.0 tends to prevent the growth of bacterial contaminants. Various salts known to be necessary for satisfactory mold growth may be added to supplement those present in the bran. The time necessary for the mold to develop sufficiently for maximum amylase production need be no more than 2 or 3 days. It is generally supposed that this point is reached just before prolific sporulation becomes evident. Conditions relative to agitation and aeration during growth are critical. The process may involve a period at rest, with no aeration, to permit rapid growth, followed by active movement of air through the mass until maximum growth is attained (57).

The mold bran or "Koji" resulting from the above process may be used "as is" for various purposes. Extracts may be made and the amylase precipi-

tated with alcohol, separated from the liquid, and dried. The water-soluble concentrates of amylase prepared by this means have wide applicability in industry. One of the better known of these concentrates is Taka-diastrase. In fact this name frequently has been used in a general sense to refer to purified concentrates of fungal amylase.

Bacterial Amylases—In many respects the techniques used for the production of bacterial amylases are similar to those prevalent in the production of fungal amylases. There is the same necessity for selecting a satisfactory strain of organism, for using a favorable medium and optimum growth conditions, and for minimizing contamination. Production does differ in two essentials: liquid media are used in which to grow the bacteria, and the pH satisfactory for active growth is close to neutral. Details of production may be obtained by consulting Effront (58), Boidin and Effront (59, 60), Schultz, Atkin, and Frey (61), and Wallerstein (62).

The media used for the growth of bacteria for amylase production customarily are high in nitrogen and low in carbohydrates. Soybean and peanut meals have been used successfully to prepare such media. They may be subjected to previous hydrolysis in order partially to break down the proteins and carbohydrates. The addition of various salts may or may not be found necessary for optimum growth. The liquid is adjusted approximately to the pH of neutrality, sterilized under pressure, then inoculated with a pure culture of the bacterial strain. This liquid customarily is held in shallow trays, the depth of the layer being only a few millimeters. Aeration is carefully regulated, and the temperature is maintained at about 30° C. When growth of the organism has resulted in the maximum production of amylase, the liquid is ready for use in such form, or the amylase may be precipitated from it by the use of neutral salts or alcohol. It is customary to remove the majority of the bacteria before precipitation of the amylase. This may be accomplished by centrifuging and to some extent by techniques of differential precipitation.

Many bacteria have been investigated with regard to their amylase-producing capabilities. The organisms of value usually have been found in the *Bacillus subtilis* group (sometimes referred to as the *Bacillus mesentericus* group). Many, perhaps most, strains of *Bacillus subtilis* appear to be deficient in this property, but occasionally a strain of great potency is isolated and used for commercial production. The economics of the production of bacterial amylases are somewhat unfavorable and, were it not for the remarkable thermostability of many of these products, it is doubtful whether they would be produced in quantity. Resistance to thermal inactivation is a property greatly desired in amylases and those produced by bacterial growth appear to be definitely superior in this respect.

C. Production of Animal Amylases—This is a phase of amylase production with which the writer has not had personal experience. The process appears to be relatively simple and only slightly different in essentials from the extraction of amylase from the higher plants. Apparently the pancreatic glands of slaughtered cattle, hogs, or horses are most commonly used. Crude preparations may

be made by drying the mass of glandular material in a vacuum and removing the fat. For products of greater purity and water solubility the fluids may be expressed by appropriate means and the amylase precipitated by neutral salts. The resulting precipitate is centrifuged, and then washed and dried to give a light colored powder of high starch-liquefying and dextrinizing powers. Activating substances such as salts or protein degradation products may be added.

Sometimes in the marketing of amylase concentrates a filler is used. Frequently this filler is a neutral salt such as sodium sulfate, but with products designed for special purposes it may be sugar, starch, or even wheat flour. Accordingly, an investigator should exercise caution when attributing properties to a certain enzyme product. For example, a product prepared by diluting a fungal amylase with wheat flour would exhibit β -amylase properties in addition to the expected α -amylase activity.

6. Other Enzymes with Amylase-like Properties. It is necessary to consider very briefly a few enzymes which have or are claimed to have functions similar to those of the α - and β -amylases discussed above.

Amylophosphatase—Mayer (63) recently has summarized the evidence which led to the postulation of this enzyme. The concept prevailed that phosphorus was intrinsically a part of the starch molecule. Upon enzymic liquefaction of starch a release of phosphorus was detected. This liquefaction apparently took place without the production in detectable quantities of either dextrans or reducing sugars. The name "amylophosphatase" was coined and the enzyme described as a "starch liquefier." The similarity between this enzyme and α -amylase, both in properties and mode of action, strongly suggests that the reaction observed was that of α -amylase with incidental liberation of phosphorus.

Phosphorylase—The researches of Cori and Cori (64) have resulted in the clear definition of a reversible enzymic system in animal tissues: glycogen + inorganic phosphate \rightleftharpoons glucose-1-phosphate. The enzyme catalyzing this reaction is known as phosphorylase. Hanes (65, 66) demonstrated the presence of a similar enzyme in pea seeds and potato tubers. Unlike the apparently irreversible amyolytic splitting of starch, the action of phosphorylase is reversible. Depending on the conditions, the same enzyme either splits starch into the hexose phosphate or synthesizes a starch-like carbohydrate from hexose-1-phosphate. The existence of such an enzyme system has pronounced physiological significance and should go far toward elucidating the nature of carbohydrate metabolism.

Amylocytase—The existence of an enzyme which hydrolyzes the supposedly very resistant outer covering of native starch granules as a preliminary action to the degradation of the internal starch was early postulated (67). The "raw starch amylase" of Blish, Sandstedt, and Mechem (68) may well be placed in this group. This was proposed as a factor present in malt and active in the breakdown of native wheat starch granules. Further evidence (24) indicates that, at least in malts, the action cannot be separated from that of α -amylase.

Glucosidases—Some of the hydrolytic cleavages apparently taking place during the action of amylase preparations on boiled starch are difficult to explain without assuming the presence of other sugar-producing enzymes. It is recognized that a maltose-splitting enzyme, maltase, frequently accompanies amylase. It seems highly probable that other glucosidases likewise are operative. α -Glucosidase is believed to play a part in the type of saccharification achieved by the action of fungal amylases on starch (28, 29). Present evidence (unpublished) indicates that part of the sugar production occurring in the later stages of the action of some α -amylase preparations may be due to enzymes other than α -amylase. For example, several α -amylase preparations from different sources were adjusted so that dextrinization proceeded at the same rate. Up to the point of loss of the iodine-staining property the production of reducing groups essentially was identical for all. The rates of sugar production following the conversion to dextrans differed markedly, suggesting either the presence of an enzyme capable of splitting dextrans and not starch, or an amylase action different from that commonly conceived.

The foregoing discussion of the amylases and of a few other enzymes with amylase-like properties is subject to modification. Unquestionably, further research will provide additional information bearing on the properties and modes of action of the enzymes we now recognize and use as industrial tools. It seems a foregone conclusion that this research likewise will lead to the discovery of new members of the amylase family and new enzymes that accompany the amylases and modify their modes of action.

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CHAPTER XVI

MODIFICATION OF STARCH BY ENZYMES

G. M. SEVERSON

1. Introduction. Enzymes are catalysts of organic origin and play the dominant rôle in almost innumerable chemical transformations. One group promotes scission of the starch molecule or of molecular starch aggregates; the enzymes accomplishing this action are designated as amylases. This chapter is concerned with the industrial application of these enzymes for inducing a modification or change in starch. The preparation and properties of amylases as well as individual applications of the several starch modifications are reviewed elsewhere in this text.

A number of physical and chemical conditions have a marked influence on the activity of these enzymes. Thus, pH, temperature, composition of the substrate, etc., all exert effects on the reaction rate or mode of reaction. For each set of conditions, there is an interrelationship between the various factors. Certain generalizations in respect to favorable or unfavorable conditions will be discussed, and pertinent specifications will be given for each of the reactions considered.

The common sources of commercial amylase or "diastatic" preparations are the cereal malts, certain species of molds or bacteria, and, to a limited extent, animal sources such as pancreatic preparations. These are discussed in Chapter XV. The enzyme products may be subjected to some purification and concentration or made with little or no treatment subsequent to cultivation, as in the case of barley malt.

It is not within the scope of this chapter to discuss the different views on enzyme classification or mechanism. On the premise that a given enzyme is restricted in its activity to inducing a specific reaction, many commercial preparations have a very complex enzyme system; others are relatively pure in one enzyme and sold for a specific type of reaction. In any case, the user must fix the conditions to favor the desired starch change.

Starch-modifying enzymes may be grouped according to the reaction they promote, as follows: (1) liquefy or solubilize starch without inducing extensive degradation of the starch molecule ("amylolytic" enzymes), (2) produce degradation products classified as dextrans with only small amounts of sugars ("dextrinogenic" amylases), (3) hydrolyze the starch to a high percentage of reducing sugars such as maltose or dextrose ("saccharogenic" amylases), (4) cleave the starch molecule by introduction of chemical groupings other than those from water (an example of which is phosphorylase), (5) cleave the starch molecule with a possible rearrangement of the units formed (an example of which is the action of *Bacillus macerans* amylase which forms the Schardinger dextrans).

The last two groups of enzymes have not as yet attracted the attention of industry, and therefore this discussion will be divided into the three subdivisions (1) liquefaction, (2) dextrinization, and (3) saccharification.

2. Liquefaction of Starch.

A. Liquefaction in the Starch Trade by Enzyme Action—Liquefaction of starch is understood to mean a disorganization of the starch granule into smaller physical units which remain more permanently dispersed than gelatinized, untreated starch. The most significant visible effect is a reduction in the viscosity or thickness of the final paste, and this should be attended by a minimum production of reducing substances such as lower dextrans and sugars.

A number of investigators believe that a definite enzyme is responsible for starch liquefaction and that selected linkages are attacked. Hanes (1) gives an interesting picture, reasoning that molecular starch chains are terminally bound by esterified phosphoric acid and that the liquefying enzyme specifically attacks this linkage. Other workers believe that the enzyme responsible for liquefaction likewise produces dextrans as commonly defined. A number of investigators (2, 3) show a parallelism between liquefaction and dextrinization under varying conditions and reason that one enzyme is responsible for both dextrinization and liquefaction.

At any rate, the enzyme component responsible for starch liquefaction is commonly referred to as α -amylase, and many excellent commercial preparations of this enzyme are available. Indeed, enzyme manufacturers seem to have concentrated their efforts in recent years on the perfection of enzymes for this purpose. This fact may reflect the growing importance of this type of starch modification in industry. Among those which might be listed as for sale in America are Amyliq, Arcy, Diastases A, BB, and E, Vanzyme, and many others possessing high efficiency.

In practice, liquefactions of corn starch are performed as follows: The starch is made up in a slurry with cold water at some convenient concentration (usually 10 parts of water to from 1 to 2 parts of starch), the enzyme is mixed in, and agitation and heat applied. About 0.1 to 1.0% of enzyme is required (based on the weight of starch treated), the amount depending on the extent of liquefaction desired and the operating conditions used. It is also desirable to adjust the pH of the conversion mixture, when necessary, to the optimal range recommended by the enzyme manufacturer, which, for the type of liquefaction described and the conditions outlined, varies from pH 5.5 to 7.0. Live steam, blown through jets into the bottom of the conversion tank, is commonly used for heating. This also supplies considerable agitation.

Common practice is to heat the conversion slurry up to a temperature in the gelatinization range for the starch as quickly as possible and then maintain this temperature until the desired reaction stage has been reached. Finally, the mixture is heated to an elevated temperature to complete the conversion, to thoroughly disperse the converted starch and finally to inactivate the enzyme. This enzyme inactivation prevents unwanted action in subsequent processing.

For corn starch, conversion temperatures vary from about 160° F. for the more thermolabile enzyme preparations up to 180° F. for those of greater thermostability. A conversion time of about 30 min. is often used and the process is completed at a temperature of 200–205° F.

Some operators prefer to heat the starch slurry slowly up to the inactivating temperature. Others claim that greater enzyme economy results from the addition of the enzyme in small amounts at various stages of the heating. However, few, if any, attempt to secure the maximal enzyme economy by first gelatinizing the starch and then cooling it to a favorable temperature for enzymic activity.

Starch-splitting enzymes act with great difficulty on ungelatinized starch, although a specific enzyme has been postulated for the action observed on native wheat starch (4). Kerr, Meisel, and Schink (5) have noted the action of malt on ungelatinized corn starch. However, the organization of the starch granule is such that liquefaction does not take place to any appreciable extent until the granule structure is weakened by gelatinization. As mentioned above, enzymes are inactivated by heat; so the two effects of the heat must be properly balanced; *i.e.*, gelatinization of the starch and enzyme destruction. Hence it may be found that a factor found optimal for the enzyme under certain conditions must be changed, in order to obtain the best results when applied under other circumstances. For example, one of the most important factors influencing converting conditions is the *pH* value. Under certain favorable conditions, such as the use of gelatinized starch at 120° F., many amylolytic enzymes show a peak activity at *pH* 5.0 to 5.5. At higher temperatures, this *pH* becomes increasingly destructive to the enzyme. At *pH* 6.5, the enzyme is more tolerant toward heat. Therefore, a higher *pH* range is used for actual plant operation than is indicated as optimum in published data, since a relatively high temperature is needed for corn starch gelatinization.

This point of the interrelationship of the various factors influencing enzymic activity constantly must be kept in mind when enzymes are used for starch degradation. There are no such values as optimal *pH*, optimal temperature, etc., except for one particular set of conditions, and with a particular starch.

These general rules may be set up.

- a. The rate of enzyme destruction increases with temperature.
- b. The higher the temperature, the faster the rate of action becomes for a given enzyme, up to the point at which enzyme destruction overbalances this effect.
- c. The higher the *pH* (up to and not exceeding 7.0), the more stable is α -amylase toward heat.
- d. The stabilities of the amylases are dependent to some extent on certain subsidiary factors; an example of this is the stabilizing action of the calcium ion on α -amylase.
- e. For the complex amylase systems, as found in barley malt, the temperature influences the mode of reaction; *e.g.*, when barley malt is used at 150° F., sugar

formation will be considerably less than if the reaction proceeds at 135° F. This is due to the difference in thermostability of the α and β components.

f. Under comparable conditions the order of thermostability of the α -amylase types is bacterial > malt > fungal.

g. For effective enzyme action, starch must be gelatinized or otherwise made accessible to the action. If the enzyme action proceeds during the course of gelatinization, it would thus follow that a starch which gels and becomes accessible to the enzyme at lower temperatures permits use of an enzyme of less thermostability.

Enzyme-converted starch is prepared as a fluid product. The resulting paste or sol costs more to dehydrate to a dry product than ungelatinized starch. To transport this modified product without dehydration from starch-manufacturing plants to its points of use, as for example in textile and paper mills, would add more to the basic cost of the starch than these industries can afford to pay. Hence this type of starch modification is usually performed at the point of use. This arrangement has worked out very well. The starch and enzyme chemists from their respective industries have worked with the customer until today many large paper and textile mills and related industries perform this modification of starch in a relatively competent fashion.

Starch manufacturers have on the market special products designed for these uses. The prime requisites in a starch for enzyme conversion are (1) that the modified starch product should be adaptable for the desired use and (2) that conversion should proceed rapidly with a minimum amount of added enzyme.

Judging from the literature, little is known concerning the improvement of starch to condition it for special use after the enzyme conversion and such modification may be difficult even though the required characteristics are known. For example, if an enzyme-converted starch is to be used as an adjunct in the clay coating of paper, the starch product should be of low viscosity, exhibit a minimum of plastic effects, and yet possess great strength in binding clay to paper fiber. As outlined by Kerr (6), these characteristics are to be found in the fractions of starch which show the highest degree of colloidal stability. This applies particularly to corn starch, since the so called linear molecules of corn starch are unstable; that is, these molecules tend to retrograde and possess low adhesive strength. As retrogradation proceeds in a clay coating paste, viscosity and plasticity are increased, and the uniform application of the clay coating becomes more difficult. The above discussion is given to show that frequently it is a fraction of starch rather than the whole starch in the liquefied state which is desired. Although effective methods are known for fractionating starch (7-9), at present the processes are relatively expensive and their use would raise the cost to or above the price of competitive products, which in the above example could be casein. However, Kerr and coworkers (5) outline a reasonably economical procedure by which it is claimed that enzyme treatment removes, partially at least, the linear fraction in preference to the branched colloiddally stable fraction.

For improvement in the ease of starch liquefaction, three approaches may be used: (1) addition of substances which promote enzyme action, often referred to as enzyme accelerators or stabilizers, (2) removal of enzyme-toxic materials, and (3) partial modification of the starch prior to the enzyme action. It must be kept in mind that any practical treatment as listed above must be of less cost in proportion to the advantage obtained than that obtained by adding a little extra enzyme to the original starch.

The literature abounds in references to so called enzyme accelerators. These substances which promote enzymic action may be grouped under four different headings which by their action (1) act as true accelerators, (2) exert solubilizing or peptizing actions on the enzyme, (3) stabilize the enzyme toward the conditions imposed, and (4) create more favorable conditions for enzymic action. Because of the vagueness of definition, the first action listed is probably more for theoretical discussion than of industrial significance. Solubilization or peptization is usually not important when industrial enzyme preparations are used under usual pasting conditions. The marked accelerating action of some of the neutral salts on pancreatic amylase might be explained on this basis. A number of compounds, both organic and inorganic, are said to stabilize enzymes, and while many of these are of doubtful significance, the calcium ion is an example of one which has found industrial application. The tolerance of α -amylase toward heat under usual conditions of production of starch pastes is definitely increased by the addition of small amounts of calcium¹ (10, 11). The most significant and perhaps only pertinent group of materials to be considered under the heading of improving conditions for enzymic activity are those substances which adjust the pH to a level more favorable for enzyme action. Thus starches to be used for enzyme conversion are usually adjusted to approximately pH 6. Inasmuch as water varies considerably in hydrogen ion concentration and because different enzyme preparations have different ranges of pH for optimal activity, the user should check the pH of his conversion mixture against the data for pH optima supplied by the manufacturer of the enzyme used.

Likewise numerous inhibitors also may be listed. The copper ion is the common one which in trace amounts can seriously retard α -amylase action.

Certain methods of weakening the granule structure, which presumably make access easier for the enzyme at lower temperatures, result in a starch which will liquefy to a given level with greater ease. These methods include dry heat treatment of starch, ball milling, and acid treatment under certain conditions.

B. Liquefaction of Cereal Mash for Fermentation—The use of amylase in making up the mash prior to cooking is a common practice in the manufacture of alcohol from cereals. The ground grain and water are mixed and the liquefying

¹ The preferential stabilizing and apparent accelerating effect of the alkaline earths, particularly calcium, on the α -amylase component of diastases and the apparent retarding effect on the β -amylase component, as well as the apparent increase in the pH for optimal activity of the amylases with an increase in the temperature of reaction have been disclosed by Kerr and Trubell in U. S. Patent App. 279,274 (1939) and in the French Patent 863,043 (1940).

agent added, usually prior to reaching the temperature of starch gelatinization. Preliminary thinning of the mash enables the operator to cook a more concentrated mash and results in savings in steam and in the costs of equipment. It also may render the mash more accessible to enzyme action in saccharification after cooking.

A satisfactory and applicable procedure is as follows: Mix the ground grain with water containing a suitable liquefying enzyme in proper concentration, in the ratio of 1 part of grain with 2 parts of water at a temperature just under that at which starch granules start to swell, and maintain it for 10 to 15 min. to allow penetration into the grain particles. The amylase must be one having good activity throughout the temperature range in which the starch gelatinization occurs. Raise the temperature of the slurry during 15 to 20 min. through the gelatinization range to 180–185° F., which is above the point of maximum viscosity, and then proceed with the cooking operations.

Corn is a common cereal used for the manufacture of industrial alcohol. Since corn has a wide gelatinization range (between 145° and 180° F.), the enzyme employed must be relatively thermostable for maximum efficiency. For this range, a bacterial preparation is superior to malt. If wheat is used as the raw material for alcohol manufacture, barley malt is very effective inasmuch as the apparent gelatinization range for wheat starch occurs at a lower temperature than for corn starch. The relatively thermolabile fungal preparations are unsatisfactory in either case.

C. Desizing—The object of desizing starch-sized fabrics is to solubilize the starch so that it may be removed by washing the fabrics. All three types of action, however, may take place; that is, liquefaction, dextrinization, and saccharification. Desizing is listed under liquefaction because both liquefaction and desizing are thought of as solubilizing actions. Cereal, bacterial, or fungal types of enzyme preparations are used.

There are many modifications used in desizing operations, and each factory has specific conditions which dictate the exact method to be employed. In essence, the cloth is treated with the enzyme, the action is allowed to take place, after which the fabric is washed. The process may be continuous or may be carried out on batches of material. A consideration of two procedures in which the enzyme action takes place will show the variance in conditions used. After application of the enzyme, the treated cloth may be pressed to remove excess enzyme and then run into a warm chamber in which the enzyme action proceeds for 30 to 60 min., or it may simply be stacked overnight, before it is washed. Gale (12) presented a discussion of desizing and also included enzymic treatments used in laundering and cleaning.

3. Dextrinization. Dextrins are degradation products of starch. During the course of shortening or cleaving the starch molecule, whether by enzymic or acid treatment, there is a gradual change in certain properties. The reducing power and the solubility increase, while the viscosity of the paste solution decreases, and starch originally staining blue with iodine gradually loses this

property. The name dextrin is assigned to the class of compounds which fall in the zone in which these properties no longer can be considered typical of starch yet before the degradation has resulted in the formation of sugars.

A. Dextrins for Adhesives—Dextrins are used extensively in the preparation of adhesives. The preparation from the raw starches is similar to that for thin boiling starch, except that starch degradation proceeds further. Enzymes from cereal, bacterial, or fungal sources are used, although the fungal ones are not adaptable for use in dextrin formation from native corn or other starches having a relatively high temperature range for gelatinization. Corn, potato, and tapioca starches are all used, depending on the special use for the resultant paste. For malt and corn starch, a conversion temperature of 150–160° F. is generally used. This temperature is quite favorable to the dextrinizing enzyme and prevents extensive sugar formation by the saccharifying component. Instead of maintenance at this temperature, heating may be continued throughout the gelatinization range. The entire amount of enzyme may either be added at once at 150° F. or as small additions throughout conversion. Higher temperatures become applicable when bacterial enzymes are used, and of course lower temperatures must be employed for fungal amylases. The enzyme usually is inactivated by further heat treatment when the desired fluidity is reached.

The dextrin paste may be dehydrated to a powder by spray-drying. Paste makers often evaporate the paste to the desired consistency and sell it as such. They also, on occasion, blend in certain amounts of torrefaction dextrins, small amounts of borax, and a plasticizer such as cyclohexanol or glycerol or just a plasticizer for making library pastes, etc.

4. Saccharification. Saccharification involves the formation of simple sugars such as maltose or glucose from starch or other high sugar polymers. This process is, of course, basic to the fermentation industry and has also become an important adjunct to the starch trade. Although two divisions are made in this section there are other important applications. For example, saccharification is vital for bread-making when flour is not supplemented with sugar for yeast food. In fact, the rôle of amylases in baking is quite complex and not confined merely to saccharification (13).

A. Saccharification for Alcohol Production—Yeast can utilize only simple sugars. Therefore starchy materials must be saccharified before conversion to ethyl alcohol. In the manufacture of alcoholic beverages, alcohol formation is not the only important consideration, and the type and amount of saccharifying agent employed is dictated not only by the amount and conditions necessary for sugar formation but by the imparted flavors, aroma, etc. Thus, from 75 to 100% barley malt is employed in brewers' mash, and it is obvious that the amount of saccharifying enzymes present is in excess of the quantity necessary in a method involving the efficient saccharification of the starch present. Individual enzyme components can produce sugars by their action on starch. Thus, β -amylase will convert starch to approximately 60% maltose. α -Amylase, in addition to producing dextrins, also produces reducing fermentable sugars.

However, complex enzyme systems are undoubtedly responsible for commercial saccharification processes in which a relatively fast and complete breakdown to fermentable sugars is required. Thus, postulated enzymes such as α -glucosidase (5) or maltase may play a very important rôle in efficient and complete saccharification of starch. A test for enzyme potency which measures only one factor (such as the Lintner value of malts) should be regarded only as an index and not as an absolute criterion of the value of that enzyme preparation in saccharification for alcohol production. The final and true value of an enzyme product for use in this type of saccharification is best judged by data of the alcohol yield. A thorough discussion of the enzyme characteristics of different amylases is given by Eric Kneen earlier in this book. The discussion following concerns processes in which the only objective is efficient saccharification and therefore is applicable more directly to the manufacture of industrial alcohol.

Barley malt is the usual enzyme source employed by the alcohol-manufacturing trade. Numerous other enzyme preparations have been used to accomplish starch conversion, notably certain species of molds. The enzyme systems of the malted cereals and the fungal preparations are both complex but differ chiefly in the relatively large percentage of β -amylase in malt and the large amount of α -amylase with none or only traces of β -amylase in the fungal preparation. Under proper conditions, both enzyme sources give efficient saccharification, and approximately the same alcohol yields are to be realized with either.

The amount of barley malt added to the cooked mash is usually 8 to 10%, on the basis of the grain weight, and the temperature is maintained at approximately 140° F. for 12 to 60 min., after which the mash is cooled to the fermenting temperature. With the usual conditions, a pH of 5 is generally considered to be about optimum. It should be mentioned that cooking adequate for a thorough disruption and dispersion of starch particles is necessary for complete saccharification. Additional enzyme action proceeds during the course of the fermentation.

While fungal preparations have not been extensively used in the industrial alcohol manufacture, there are numerous references to the use of this type of amylase. Renewed interest in the application of fungal amylase (in particular, the use of *Aspergillus oryzae*) has resulted from the work of recent investigators (Fulmer, Underkofler, and others (14-16) and Beresford and Christensen (17)) who have studied optimal conditions on the basis of resultant alcohol yields from various common starchy materials.

The mold can be satisfactorily grown on wheat bran, and from 2.5 to 6% of this material is adequate for maximum alcohol yields. The use of the lower amount is satisfactory if the mash has been quickly cooled according to the method as outlined by Beresford and Christensen (17). This claim is substantiated by Underkofler (18). The enzyme may be added at 130° F. during the course of cooling the mash or at the fermentation temperature. The optimal pH is approximately 5.0. The chief advantages of mold bran as advanced by the above investigators are the lower cost of saccharification and as good or better alcohol yields in comparison to those of barley malt.

B. Production of Enzyme-converted Sirups—Starch-hydrolyzing enzymes also are used in the preparation of sugar sirups. Distinguishing features of sirups of this type are sweetness, high fermentability of dry solids, and stability toward crystallization in spite of high sugar concentration. These effects are due, in part at least, to the total high sugar concentration of which the proportion of maltose is relatively high.

A method for making these enzyme-converted sirups from corn starch is, according to Kerr and Schink (19), as follows:

A starch suspension is made up to 12° Bé. and converted under 30 lbs. of steam pressure until the "purity" (dextrose equivalent, calculated from the reducing value) reaches about 57. The converted material is then clarified, and the acidity is adjusted to about 5.0 and the temperature to 130° F. A malt diastase preparation of high Lintner value is then added (at 250° Lintner about 0.5% enzyme, on a dry solids basis, is necessary). Conversion is continued until no further increase in the "purity" is noted. A limiting "purity" of about 64 is usually attained. The liquors are then clarified and concentrated to the desired content of solids, usually about 42° Bé. In all of the finishing operations it is necessary to control the pH and temperature carefully, so as to secure the proper sugar balance and the maximum results from clarification treatments.

Other conditions can be used (20). For example, a starch suspension of higher density can be used which is converted to a sirup with a "purity" of 50. For products with lower final values for the dextrose equivalent, it is permissible to use higher initial starch concentrations, as reversion of dextrose to gentiobiose depends on the dextrose concentration. The products of acid conversion are submitted to a clarification treatment, concentrated to 30° Bé., cooled to 135° F., adjusted to pH 6.5, and the solution is converted with a fungal type of amylase to the desired sugar content. Clarification and final concentration follow.

There are many other important applications for enzyme preparations than those outlined above. Since enzymes are more or less specific in their action and control is relatively simple, their value and worth are becoming increasingly apparent. The fundamental research to which so many workers have contributed has made possible the notable advances in this field.

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CHAPTER XVII

MISCELLANEOUS REACTIONS

LLOYD M. COOKE AND RALPH W. KERR

Starch reacts or appears to react with several types of reagents other than those that have already been considered. At present, only one of these has any technical significance worthy of note, but it is quite possible that the application of the fundamentals involved in all will occur in the future. Of the remaining reactions that have not been discussed, those of starch with iodine, aldehydes, and with gaseous hydrogen are the most interesting.

1. Reaction of Starch with Iodine. One of the earliest observed and one of the most characteristic reactions of starch is its ability to form an intense coloration with iodine. Most frequently the color is blue, but on occasion shades through purple to red are observed. Many theories have been proposed to explain this effect which was observed early in the development of starch chemistry when the experimental background was inadequate for anything more than a guess. Recent research has clarified the situation, materially, and has offered an intelligent and logical picture of the mechanism of the reaction.

Apparently the discovery that the addition of iodine to dilute starch solutions results in a blue coloration is attributed to de Claubry (1). In 1814, he reported that the reaction is characteristic, regardless of the source from which he obtained the starch. Subsequently it was observed by Leroy (2) that water was necessary for the reaction to take place. This conclusion was substantiated by Stocks (3) who studied the effect of iodine vapor on starch. Stocks, as well as Mylius (4), Roberts (5), Hale (6), and others of this period, believed that hydriodic acid, or an iodide, was the active reagent. Roberts supported his contention by showing that pure iodine in chloroform, free from hydriodic acid, gave no blue color when added to starch, but that if the solution was heated or if an acid was added, the blue color was formed. Harrison (7) found that certain salts aided the reaction.

Pickering (8) made the observation that the blue, starch-iodide coloration disappeared when the solution was brought to the boiling point but reappeared when the solution was cooled. The temperature at which the color became

apparent again was not fixed, but varied with conditions such as the concentration of starch and iodine. These results have been confirmed by Vogel (9), Radley (10), Phol (11), and others. Phol found that the intensity of the color which appeared increased as the temperature decreased, and drew the significant conclusion that hot water has a greater affinity for starch than has iodine but that iodine has a greater affinity for starch than has cold water. Another significant conclusion, drawn by early workers, is that of Payen (12, 13), who stated that the loss in blue coloration on heating is due to an expansion of the starch aggregates. On cooling, the aggregates assume their original form and the blue color reaction can again take place.

Acids and certain salts, particularly potassium iodide and sodium sulfate (14-16), have been found to increase the sensitivity of the reaction. Alkali, chloral hydrate, and certain other substances can inhibit the reaction (17, 18).

To account for the blue color, some have assumed an adsorption phenomenon in which colloidal aggregates were involved, others have postulated a complex formation with starch molecules, while still others have assumed that a definite compound was formed. Especially during periods when starch was believed to consist of only one molecular species, has the latter theory been advocated.

Among the earlier workers who considered that a definite compound was formed were Schoenbein (19) and Rouvier (20). Rouvier reported that a series of compounds was formed according to the general formula $(C_6H_{10}O_5)_{18}I_n$, where n increased from 2 to 5 as the amount of iodine added to the starch was increased. The actual amounts of iodine found in these compounds varied from 3 to 19.6%. Toth (21), however, obtained starch iodides containing as high as 22.8% iodine by dissolving starch in hot glycerol and precipitating the compound by the addition of a solution of potassium iodide and iodine. Neither these nor other workers apparently agreed on the combining proportions between iodine and the "starch molecule." Padoa and Savarè (22) found 19% iodine in the compound, while Andrews and Goettsch (23) obtained two different compositions, one corresponding to the formula $(C_6H_{10}O_5)_{12}I$ and the other to $(C_6H_{10}O_5)_{12}I_2$. Von Euler and Myrbäck (24) found two compounds also but gave as formulae $(C_6H_{10}O_5)_{18}I_2$ and $(C_6H_{10}O_5)_{18}I_4$.

The adsorption theory gained considerable support from the work of Lottermoser (25, 26), who obtained typical adsorption curves. His measurements were based on the determination of an electric potential in the system, free iodine/iodide ion, as formulated by the Nernst equation, and also on the distribution ratio of free iodine between 0.1 *N* KI solution and CCl_4 . The electric potential measurements especially should be called to attention, since recent work based on potentiometric measurements has proved of great value in a clarification of the problem.

Other methods of physical chemistry employed in such studies have been those for determination of osmotic pressure (27) and freezing point depression (28), colorimetric methods (29), and conductivity measurements (30).

It is believed significant that Berzeller (31) found that starch adsorbed no iodine from solutions of iodine in CCl_4 and found that the amount adsorbed from alcohol was less than that from water.

Again, the heterogeneity of starch seems to have received little attention in explanations advanced for the adsorption phenomena. Hanes and Cattle (32), for example, concluded that the starch molecule contains groups along the molecular chain which can adsorb iodine to give the characteristic coloration of starch. They considered that, as hydrolysis of the molecule by β -amylase proceeds, there is a progressive decrease in the number of adsorbing groups, which decrease accounts for the resulting changes in color from blue to purple to red. Bergmann and Ludewig (33) suggested that the oxygen atoms forming the bridges in starch are the centers of adsorption and this may explain the gradual change in color from blue to red as starch is progressively degraded. More will be said in this connection in subsequent sections, since these theories do not seem to be in accord with the facts.

A significant advance toward a better understanding of some of the fundamentals of the iodine reaction was made by the development of the spectrophotometer and by its use in work reported by Simerl and Browning (34), who showed that all of the industrial starches studied gave a blue coloration with iodine, the color of which resulted in a minimum of light transmission at a wave-length in the neighborhood of $600\text{ m}\mu$. The starch concentration did not affect the position of the minimum in the light absorption curve. They found also that except at certain limiting concentrations of iodine, Beer's law held; that is, the log of the fractional transmission was proportional to the starch concentration. A straight line relationship was obtained over the range 5 to 95% transmission by plotting the log of the percentage transmission against the concentration of corn, potato, tapioca, wheat, rice, rye, and canna starches. An iodine concentration of 0.1 g. per liter and a KI to I ratio of 1.5 : 1 by weight was used. They found that the blue iodine coloration was produced by that fraction of corn starch which was precipitated in electrosedimentation of the paste, which they called α -amylose, in accordance with the nomenclature used by Taylor. It is undoubtedly a fraction of the linear components of corn starch, or an amylose, as it is more conveniently called. This fraction gives an exceptionally low, minimum light transmission at about $600\text{ m}\mu$. The non-sedimenting (or branched) fraction of corn starch (β -amylose, according to Simerl and Browning) shows a very high light transmission at wave-lengths between 600 and $700\text{ m}\mu$ and the transmission falls off as the wave-length is decreased to $400\text{ m}\mu$.

Considering the fractionation technique employed (which would probably give the opposite results with potato starch), it is remarkable that these workers should have obtained such clear results in demonstrating that the fraction which we now believe contains predominantly linear configurations should be the constituent colored blue by iodine. So sharp were the results, that one might use the data of Simerl and Browning to estimate the relative proportions of the predominantly branched and the predominantly linear fractions of corn starch.

The proportions calculated from these results would be 73 and 27% respectively, which are surprisingly close to values obtained by other means.¹

Kerr and Trubell (35) have checked and extended the above conclusions by the use of extensively purified starch constituents. With pure, crystalline amyloses obtained from corn, potato, and tapioca starches, it has been found that in all cases the minimum per cent of light transmission occurs in the same range of wave-length; that is, in the neighborhood of 600 to 620 $m\mu$. By plotting the log of the per cent of light transmission against the concentration of amylose,

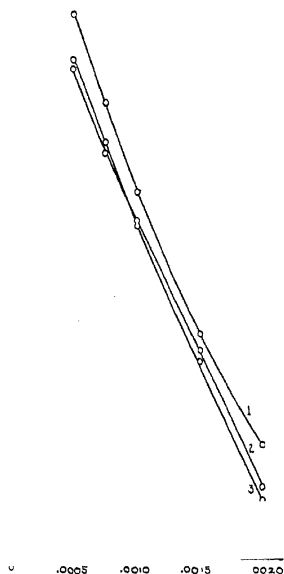


Fig. 86. Log per cent light transmission (ordinate) versus per cent concentration (abscissa) for crystalline amyloses from (Curve 1) corn starch, (Curve 2) potato starch, (Curve 3) tapioca starch.

Figures 86, 87, and 88: Reproduced through the courtesy of Paper Trade Journal.

fairly straight lines are obtained over a considerable portion of the range of light transmissions studied, as is shown in Fig. 86. As might be anticipated, the purified corn constituent shows the most noticeable variation from Beer's law, particularly at higher concentrations, whereas the tapioca amylose, being the most stable colloiddally, gives the most nearly linear relationship. For some reason not entirely clear, the slopes of the three curves are all slightly different, and the crystalline amyloses of both potato and tapioca give slightly lower minima for per cent of light transmissions per unit weight of amylose than does corn, crystalline amylose. However, considering the wide difference in the relative chain length of the corn amylose compared to those of tapioca and potato, it would seem safe to conclude that, above a certain minimum chain length, the function, log of the per cent of light transmission : concentration, is not materially affected by a wide variation in the molecular magnitude of the amylose. Chain lengths less than about 25 glucopyranose units give very weak colorations, since Naegeli's amylopectin² with iodine gives a relatively high percentage of light transmission even at 600 $m\mu$.

Purified amylopectin fractions, that is fractions of more complexly constituted molecules of starch, when freed from the last traces of essentially linear chains, as for example, by fractional precipitation with alcohols, give a reddish color with iodine and a high percentage of light transmission in the wave band 600 to 700 $m\mu$, as is shown in Fig. 87.

¹ See Chapter VIII and the sections that follow.

² See Chapter VIII.

"Intermediate fractions," of corn starch at least, do not give abruptly occurring minimum per cent light transmissions in any other visible wave band than that of the amyloses with iodine. By "intermediate fraction" is meant one which is obtained by the addition of butanol or limited amounts of butanol

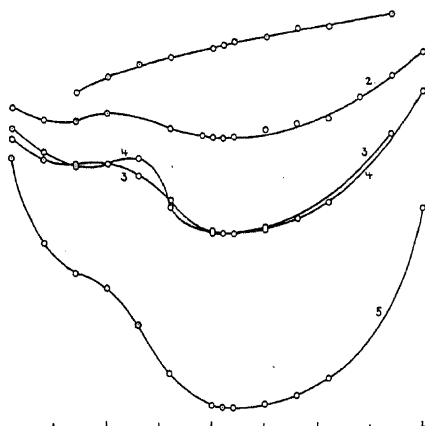


FIG. 87. Per cent light transmission (ordinate) *versus* wave-length in millimicrons (abscissa) for corn starch and its fractions at 0.002% concentration. Curve 1, highly purified "amylopectin" fraction; Curve 2, intermediate fraction (β -amylase conversion limit, 60%); Curve 3, defatted corn starch; Curve 4, mixture, 30% crystalline amylose, 70% "amylopectin;" Curve 5, crystalline amylose of corn.



FIG. 88. Log per cent light transmission (ordinate) *versus* percentage composition (abscissa) or known mixtures of crystalline amylose (lower scale) and amylopectin (upper scale) of corn at a total concentration of 0.002%. The solid circle is for whole corn starch.

and methanol to solutions or dispersions of the residual starch which remains after removal of the most simply constituted chains from starch, such as for example, by extraction with water at 70° C. The transmission curve obtained for one intermediate fraction is shown in Fig. 87, Curve 2. It is obtained by saturating a corn starch dispersion with butanol, supercentrifuging the precipitable fraction, adding in all 14.5% butanol and 13% methanol, by volume, to the reheated centrifugate, and recrystallizing the second precipitate (which forms on cooling) several times from hot water to which the same ratio of butanol and then methanol is added. This fraction represents nearly 50% of the total weight of corn starch and exhibits a limit of conversion to maltose by β -amylase of 60%.

The results reviewed might be interpreted to mean that no fractions exist in starch intermediate in structure between a linear one and one that is branched according to a definite pattern. They also might be interpreted to mean that if intermediate variations in structure exist and if sections of these molecules are sufficiently linear and of sufficient length they too may give a coloration with iodine which results in a minimum light transmission at a wave-length of approximately 600 m μ .

The results obtained by spectrophotometry with various known combinations of pure, corn crystalline amylose and a highly purified and possibly most complexly constituted fraction of corn starch are shown in Fig. 88. A total carbohydrate concentration of 0.002 g. per 100 cc. was used in all cases. The solutions were made by dissolving the products in dilute NaOH, acidifying the solutions to pH 5.0 with HCl, and diluting to half the final volume. An equal volume of a solution of iodine in KI was then added of such concentration that a final ratio of KI to iodine to starch of 1.5 : 1 : 1 resulted. The log of the per cent transmission was determined with a Coleman, Model 10-S, spectrophotometer and the results obtained were plotted against the per cent "amylose" and the per cent "amylopectin" known to be present in the various mixtures. On the assumption that only two types of configuration exist in corn starch, linear and branched, and on the assumption that any variation in the two types, respectively, in starch, does not materially alter the function, log per cent light transmission corresponding to carbohydrate concentration, the results in Fig. 88 may be used to estimate the relative proportions of each type in corn starch. A value for the per cent light transmission was obtained for whole corn starch, similarly solubilized and at a like concentration stained with iodine. This value was placed on the curve in Fig. 88 and is the solid dot shown. This point corresponds to a percentage composition of 25.5% "amylose" and 74.5% "amylopectin" for whole untreated corn starch. Corn starch, defatted by refluxing four successive times with 85% methanol at the boiling point for 4 hrs. in all gives values of as high as 29% of "amylose."

The per cent light transmission at various wave-lengths for defatted corn starch and iodine are also plotted in Fig. 87. It is similar in shape to one obtained for untreated corn starch, but the lower light transmission values obtained with

the defatted starch may be explainable by the possibility that the fat present may affect the dispersion of the linear chains of the untreated starch or by the consideration that the fat probably affects the ability of the amylose to orient with iodine.

With the same technique of alcoholic fractionation to prepare the end members, or fractions of both potato amylopectin and tapioca amylopectin, and by use of the corresponding crystalline amyloses with these products, on application of the spectrophotometric method described for corn starch it was found that potato starch contains 25.5% "amylose" and 74.5% "amylopectin" and that tapioca starch contains 20% "amylose" and 80% "amylopectin." Waxy maize starch was examined with iodine and found to give 94.1% light transmission at a wave-length of 610 m μ , with a starch concentration of 0.002%. This value is of the same order of magnitude as the per cent light transmission for the best amylopectins. Daffert (36, 37) first reported that the starch from glutinous rice, grown in China, gave a red or reddish brown coloration with iodine, and this abnormal result has been confirmed by many workers. The term waxy has been applied to this starch. When the waxy genetical characteristics are crossed into such grains as corn, barley, and sorghum, the starches produced by the plant also stain a reddish color with iodine. Since it is now known by several independent methods that all of these waxy starches and glycogen are practically devoid of perfectly linear chains,³ the reason why they give an abnormal coloration is apparent.

Curve 4 in Fig. 87 shows the values for light transmission at various wave-lengths when a combination of 30% corn crystalline amylose and 70% purified corn amylopectin, at 0.002% total concentration, is colored with iodine. The curve has a shape similar to but not collateral with the one for corn starch, Curve 3. The intermediate corn starch fraction (the results for which are shown in Curve 2) gives approximately the same diastatic analysis as corn starch. Its β -amylase "conversion limit"⁴ to maltose after three recrystallizations from a hot mixture of 100 parts, by volume, of water, 20 parts of butanol, and 18 parts of methanol was 58.7, 60.9, and 59.6%, respectively. It should, therefore, be composed of the same proportions of amylose and amylopectin as the parent starch, if, indeed, there are only two structurally different components in the starch. When the spectrophotometric method of analysis and the results for the known mixtures plotted in Fig. 88 are used, this intermediate fraction, however, shows only 13% "amylose." Obviously, either the method is not entirely adequate, or else it is based on assumptions which are not entirely valid.

It should be pointed out that Maquenne and Roux (38) observed many years ago that amylose (the fraction completely converted to maltose by diastase) is the starch component responsible for the blue coloration of the starch with

³ A small quantity of material crystallized by butanol can be obtained from some waxy starches by extraction with hot water, concentration of the extract, and saturating the concentrate with butanol.

⁴ Refer to Chapter VIII for the definition.

iodine. Indeed, these investigators attempted to apply this observation to a colorimetric estimation of the amount of amylose in starch. Their early results were too high, since it was assumed that their hypothetical component, amylopectin, gave no color with iodine. It is unfortunate that no better method for evaluating colors was available during this period. It is also unfortunate that in a comparison of the intensity of amylose-iodine colors with that for whole starch the data of Maquenne and Roux should have checked so well with their diastatic analysis, showing starch to contain 80% amylose. This erroneous conclusion concerning the composition of starch, upon which many of the theories

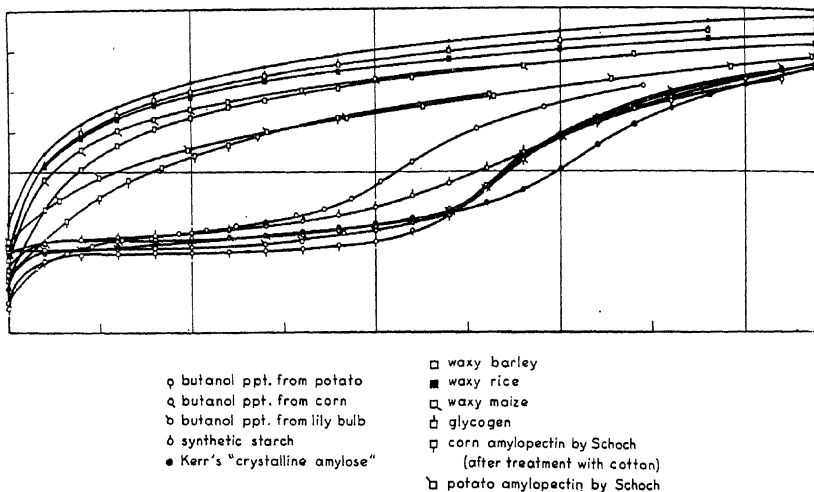


FIG. 89. Titration of amylose and amylopectin materials. The uppermost curve is a titration of 0.05 *N* potassium iodide. Ordinates: E.M.F. against normal calomel half cell, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26. Abscissa: cubic centimeters of 0.001 *N* iodine solution, 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0.

Figures 89, 90, 91, and 92: Reproduced through the courtesy of J. Am. Chem. Soc.

of starch chemistry during the following three decades were based, must be definitely discarded.

The reaction of starch with iodine very recently has been critically studied by four investigators, French, Rundle, Baldwin, and Bates. A series of four papers which may very well prove to be classics in this phase of starch chemistry may be included in this discussion. Bates, French, and Rundle (39) studied the iodine reaction potentiometrically with various starches, glycogen, and also fractions of amylose and amylopectin, among which were "synthetic starch" prepared by Hassid and McCreedy (40), the total butanol-precipitable and non-precipitable fractions of corn and potato starches prepared by Schoch (41), and the corn, crystalline amylose of Kerr and Severson (42). Their method of inspection is to disperse thoroughly the starch sample in dilute KOH, neutralize to methyl orange with HI, and then titrate the sample with a standard 0.001 *N*

iodine solution in 0.05 *N* KI. The titration is followed in a Leeds and Northrup, type K, potentiometer, with a bright platinum electrode. The E.M.F. developed against a normal calomel half cell is plotted against the cubic centimeters of iodine added. The results of several titrations are shown in Figs. 89 and 90. It is apparent that branched structures, such as amylopectins, glycogen, and the

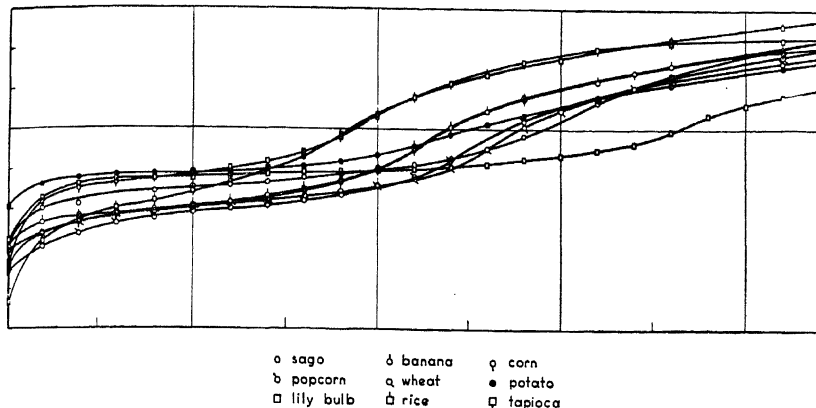


FIG. 90. Titration curves of some whole starches: 100 cc. of a 0.04% solution. Ordinates: E.M.F. against normal calomel electrode, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26. Abscissa: cubic centimeters of 0.001 *N* iodine solution, 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0.

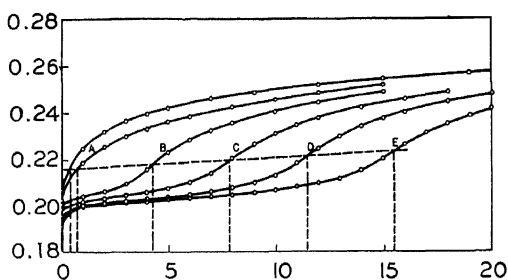


FIG. 91. Titration of mixtures of amylose and amylopectin. Curve A, 0.01 g. of amylopectin from potato starch; Curve B, 0.0075 g. of amylopectin from potato starch plus 0.0025 g. of "crystalline amylose;" Curve C, 0.0050 g. of amylopectin from potato starch plus 0.0050 g. of "crystalline amylose;" Curve D, 0.0025 g. of amylopectin from potato starch plus 0.0075 g. of "crystalline amylose;" Curve E, 0.01 g. of "crystalline amylose." The uppermost curve is for a 0.05 *N* KI solution. Ordinate: E.M.F. against normal calomel electrode, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28. Abscissa: cubic centimeters of 0.001 *N* iodine solution, 0, 5, 10, 15, 20.

waxy starches, give a characteristically smooth curve which follows the control curve for iodine alone quite closely in shape. On the other hand, linear or amylose fractions show a break in the curve, forming a definite step. Starches or mixtures containing linear chains show a point of inflection also, and this point, projected to the ordinate for iodine additions, gives a value which is proportional to the amount of pure, corn crystalline amylose in known mixtures

of the amylose and purified amylopectin from potato, as shown in Fig. 91. The results obtained with various whole starches are then used as a basis to calculate the proportions of "branched" and "linear" fractions in each starch. The method is further elucidated in the discussion which follows. By this method the waxy starches (rice, sorghum, corn, and barley) and glycogen show no linear constituents; tapioca and rice starches, 17%; banana, corn, potato, popcorn, and wheat starches, values between 21 and 24%; sago starch, 27%; and lily bulb starch, 34%.

Amylopectin fractions appear to take up a very small amount of iodine by absorption or adsorption, but the amylose fractions take up a considerable amount of iodine with a small rise in the potential until the point of inflection is reached. Here the complex formation has become completed and except for small amounts of iodine taken up by another mechanism, possibly also an ad-

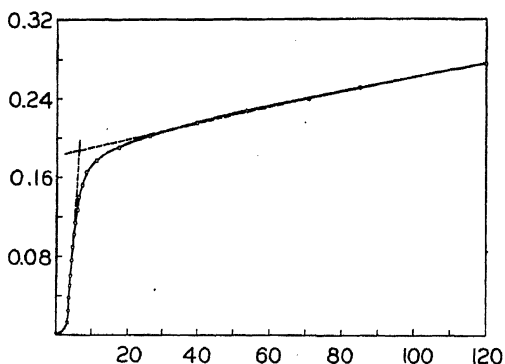


Fig. 92. Amount of iodine bound by "crystalline amylose" from corn, as a function of the iodine concentration. Ordinates: grams of iodine bound per gram of amylose, 0, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32. Abscissa: iodine concentration (moles per liter $\times 10^{-3}$), 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120.

sorption, the potential increase then becomes more nearly the usual function of the iodine additions, by themselves. A more accurate idea of what is occurring at the inflection point may be obtained by plotting the amount of iodine bound per gram of carbohydrate against the concentration of free iodine in the solution. The concentration of free iodine, corresponding to any potential, may be calculated by using the curve for 0.05 *N* KI. This value is subtracted from the total iodine present to estimate the bound iodine for any given addition of iodine. The results of this calculation are shown for the crystalline amylose of corn in Fig. 92. It will be observed that the percentage of bound iodine increases sharply until finally in the neighborhood of 18 to 20% the curve breaks, thus indicating the completion of complex formation.

Of the linear materials, Kerr's corn, crystalline amylose takes up more iodine per gram by complex formation than any other. It is concluded by Bates, French, and Rundle that in view of this and because the product shows the best

crystalline properties, as discussed subsequently, it is the purest and all other amyloses are therefore contaminated to some extent with the amylopectin fraction.

The authors and their associate, Dr. Schoch, have substantiated the latter statements in that they find the percentage of iodine bound by the corn, crystalline amylose to be 20.0% by a slight variation in the technique given above.⁵ Moreover, pure potato and tapioca crystalline amyloses, as prepared by one of us, were found to give identical values: potato 20.1%, and tapioca 20.1%. It would appear that slight differences in the constituent molecules of the amyloses, respectively, do not affect the percentage of iodine which they can bind in complex formation. It would also appear that the method described may be used as a criterion for the purity of "linear" fractions.

The theory of the mechanism by which the iodine reacts is developed in three communications. French and Rundle (43) established that the Schardinger α -dextrin is a ring-shaped molecule, about 8 Å. thick and 14 Å. in diameter. The molecule contains 6 glucopyranose units, mutually joined through 1-4 α -glucosidic links. It has a 2-fold axis of symmetry. The dextrin forms blue addition compounds with iodine, which must be closely related to the starch-iodine complex. Considerations of packing and symmetry indicate that in the crystalline form the iodine molecules must be placed within the dextrin rings with the long axis of the iodine molecules along the 2-fold axes of the dextrin molecules. The resulting structures consist of tubes formed of rings of Schardinger dextrin with iodine molecules arranged linearly down these tubes. The dichroism exhibited by the dextrin is in agreement with the structure suggested. Rundle and Baldwin (44) reported that starch-iodine solutions are dichroic during flow. The dichroism indicates that the long axes of the iodine molecules and the molecules of the starch which react are parallel. Rundle and French (45) find that, whereas granular and retrograded forms of the "linear" amylose fractions undoubtedly have extended chain configurations, the crystalline forms which give a V type x-ray diffraction pattern, such as the crystals of Kerr and Severson, form helical coils. The crystalline platelets prepared by Kerr are substantially uniaxial. On edge they are highly birefringent and when a red plate is used it is noted that the retardation of light with its electric vector parallel to the surface of the platelet is greater than the retardation of light with its electric vector normal to the largest surface. It is concluded that the helical coils are arranged with their axes normal to this surface, so that looking at this face, one would see the hollow interior of tubes formed by these coils as shown diagrammatically in Fig. 93. These crystals, unlike native starch or retrograded fractions, will absorb iodine vapor, and the x-ray diffraction pattern is not materially altered

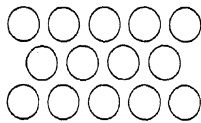


Fig. 93. Diagrammatic sketch of a section of the largest face of crystals of corn amylose, showing the projection of helical coils.

⁵ As suggested by Dr. Schoch, HCl was used instead of HI for neutralization and the Coleman electrometer was used.

by such treatment. Stained with iodine in this manner, the crystals are extremely dichroic on edge, which fact indicates that the long axes of the iodine molecules are parallel to or coincident with the axes of the helical coils. Therefore, the coloration results from the entrance of the iodine molecules into these tubes, made by the helical coils, in a manner analogous to their entry into the tubes formed by the rings of the Schardinger dextrin, which are stacked one on top of the other in the dextrin crystal.

It is believed that the blue coloration which results when iodine is added to a solution of amylose is produced because these long extended chains are induced to coil around the iodine molecules. It would seem that when heat is applied the coils tend to extend themselves; the association of iodine within the helices is broken and the color disappears.

Earlier, Freudenberg (46) found that it is possible to construct models of helical starch chains which have essentially hydrocarbon linings. He suggested that such helices would be capable of holding linearly arranged iodine molecules which should produce a blue color. The work reviewed is a confirmation of this mechanism which was proposed from theoretical considerations. Freudenberg suggests that the hydrocarbon linings of these helical rings hold the iodine by lipophilic association, and hence the absorption bands are influenced just as they are when iodine is dissolved in certain organic solvents. It is to be noted, furthermore, that the hydroxyl groups are located on the exterior of such coils. As pointed out by Freudenberg, external hydroxyl groups probably account for the extraordinary crystallizing property of large polysaccharide rings such as the Schardinger dextrans. The crystallizing property of the amyloses when they are induced to coil by some additive such as butanol, discussed elsewhere, may be explained on the same basis.

From more recent research by his group, Foster (47) proposes that the stability of the amylose-iodine complex is due to resonance interaction between the iodine molecules oriented in the amylose helix, rather than to forces between iodine and amylose. The blue color is thought to be due to a shift in the absorption frequency caused by resonance between the iodine molecules in much the same manner as chains of conjugated polydienes behave as linear harmonic oscillators which shift the wave-length of the absorption maxima by a value which is a function of the number of units conjugated. On this basis, it might be expected that the longer the helix, the more iodine molecules could be oriented end to end, which might also be expected to produce an effect of increasing the mass of the oscillator, proportionately. The shift in wave-length which leads to the blue color as the amylose chain length is increased is qualitatively explained. A slowing of the characteristic vibration frequency would probably have the effect of stabilizing the complex (resonance stabilization), and data are correlated to show the dependence of the affinity for iodine (characteristic potentials) on amylose chain length according to this view-point.

However, the effect of the van der Waals forces between iodine and amylose cannot be neglected entirely, since it is necessary to account for the formation

of helices by the addition of iodine to a solution of extended chains of the amylose.

2. Reaction of Starch with Formaldehyde and Other Aldehydes. The action of aldehydes on starch is probably quite complex and is not thoroughly understood at present. It is quite likely that the reaction proceeds by several mechanisms, or stages, depending on the concentration of the particular aldehyde used, the extent to which the reaction is permitted to take place, and other conditions, such as the acidity and the alkalinity of the medium and the addition of other catalysts. Aldehydes appear to react differently with starch in the presence of strong alkali and in the presence of ammonia and urea. This discussion is not primarily concerned with these latter reactions but it will treat mainly of the less complex system, aldehyde and starch.

If a starch paste is treated with a 40% formalin solution, it will be observed that the ability of the starch to stain blue with iodine will gradually disappear, the extent depending on the amount of formaldehyde added. The color with iodine changes from a blue, through purple, to red, and finally to yellow, which indicates the final disappearance of the coloration. This effect is so strikingly similar to the action of acid and enzymes in degrading starch through the various dextrin stages, that early workers, such as Woker (48) and Maggi (49), were led to believe that the action of formaldehyde is a hydrolytic dextrinization. The theory was found to be weak, for Maggi noted that the viscosity increased during the reaction and that the specific rotation of the solution did not decrease proportionately to the color change, as is noted in reactions in which hydrolytic agents are used. Maggi suggested that the aldehyde can degrade starch only to a dextrin stage and that these dextrans exhibit a decidedly different volume contraction from that of the diastatically made dextrans.

Syniewski (50) proposed that the hydrolytic action of the formaldehyde might be a carbinol hydrolysis, which accounts for the fact that the end-products do not reduce Fehling's solution.

Jacoby (51) showed, however, that the conclusions drawn from changes in iodine coloration were not valid, since after starch has been allowed to react with aldehyde until it has lost its ability to produce a color with iodine, the coloration may be progressively restored by the addition of ammonium acetate, whereby the aldehyde is removed from the medium (and possibly also the starch) to form hexamethylenetetramine. That little degradation in the starch has occurred during the formaldehyde reaction is confirmed by the work of von Kaufman (52, 53). If alcohol is added, the starch is precipitated in the same state as it is obtained by adding alcohol to untreated starch pastes. With mild conditions for the reaction of formaldehyde, such as treating whole starch with 40% formalin at room temperature, and provided the reaction has not been too extensive, the iodine coloration given by starch may be restored simply by the addition of large quantities of water. In other cases, the addition of an acid will facilitate the ability of the product to form a blue color with iodine (50).

Woker attempted to defend his theory by suggesting that a resynthesis of the starch degradation products occurs under certain conditions, which synthesis may have occurred during the experiments of other workers (54, 55). However, Samec and Mayer (56) could find no evidence for a degrading or resynthesizing action. The reaction was studied under various conditions with starch and its amylose and amylopectin fractions. In all cases, a highly hydrated compound (or complex) is formed, which finally gives no color with iodine and which is of the same order of molecular magnitude as before treatment. For starch so treated the dialyzable fraction remains about the same, but the viscosity is higher and the optical rotation is only slightly lower than that for the original starch. The conclusion was drawn that a loose addition product is formed. Samec also made the significant observation that the increase in viscosity which results from the addition of formaldehyde reaches its peak, in all cases, at the point where the iodine coloration disappears.

Samec and other workers have likened the action of formaldehyde on starch to that of alkali. Samec has noted that, if potato starch is treated with formaldehyde freshly distilled over CaCO_3 , the granules swell and form an exceptionally thick gel. The volume of the gel constantly increases until it finally distributes itself completely throughout the liquid to produce a very viscous solution.

If aldehydes of less activity, such as acetaldehyde, are added to starch solution, on aging there is no loss in the ability of the sol to form a blue color with iodine, which loss otherwise normally occurs. But if the acetaldehyde is removed, the normal decrease in such ability begins at once. Normally, starch solutions lose their ability to color blue with iodine owing to a retrogradation effect. The linear chain molecules become intramolecularly cross-bonded, possibly by hydrogen bonding, and it thus becomes more difficult for these chains to assume a helical shape around the iodine molecules, as described in the previous section. That the action of aldehyde is to prevent retrogradation was shown by Samec (57). Whereas 1% solutions of amylose change within a short time to milky liquids and at higher concentrations quickly coagulate, 1% amylose solutions remain clear and stable in the presence of added formaldehyde. With a sufficient concentration of the latter, however, the amylose loses its ability to color blue with iodine.

The more advanced stages of the starch-formaldehyde reaction have been studied by Classen (58). Starch treated with formaldehyde at higher temperatures produces finally a compound from which the excess aldehyde may be removed by boiling in water or by washing the product with a solution of sodium bisulfite. The starch product is insoluble in the hot water and hence may be isolated. It shows a constant composition of 1 mole of formaldehyde to 1 mole of starch and remains stable even when heated to 180°C .

From the available facts, the mechanism of the action of aldehyde on starch might be outlined as follows. In the initial stages of the action the aldehyde may enter into the original orientation within the granule to form a complex with hydroxyl groups where hydrogen bonding is weakest or where it does not

exist. As the reaction proceeds, the molecular structure so opened up tends to swell, since, after a sufficient number of hydroxyl groups have become associated with the aldehyde, the network of the molecules becomes enlarged and in addition water molecules can now enter into the interiors of these orientations. If the starch has been pregelatinized, as by heating in water, and the network is opened up through association with water molecules, then it would seem that some of these water molecules either are replaced by the formaldehyde molecules or that the hydroxyl groups of the carbohydrate associate first with the aldehyde and then perhaps with the water present. Evidently only by a "blocking" effect at these hydroxyl groups could the direct association of linear chains (or linear sections) be prevented as the paste ages. As the linear chains become loaded with aldehyde groups, their ability to form helical coils around iodine molecules is decreased, and the intensity of the blue coloration with iodine is accordingly decreased also. The three-dimensional network of the branched chains becomes opened and they decrease in their ability to absorb and hold iodine within their interiors. The type of association discussed is evidently weak and easily reversible, but, as the reaction proceeds, it seems probable that a more stable bond is formed. Possibly cross-bonds are formed from a hydroxyl group on one chain through a methylene group to a hydroxyl group on another chain. This would increase the apparent viscosity⁶ and in some cases the gelling properties of the system and decidedly lessen the tendency of linear chains to orient with iodine in much the same manner as direct cross-bonding of hydroxyl groups accomplishes the same results in the early stages of retrogradation. Finally, the number of cross-bonds through methylene groups increases to such an extent, per carbohydrate molecule, that they become relatively stable, as are the highly retrograded linear fractions of untreated starch. They lose their property of gel formation, become quite insoluble in hot water, and may be heated to 180° C. without decomposition. Treatment with acid is then required to liberate the aldehyde. It would be most interesting to isolate, if possible, and fully characterize the carbohydrate which is liberated at this point. Treatment with dilute alkali is sufficient, however, to open up these resistant structures, as it is also for retrograded amylose except that in the extreme stages of retrogradation. In the presence of dilute alkali the aldehyde becomes liberated as the starch is regenerated. Another type of reaction may then follow, particularly if the solution is heated.

Some of the applications of the aldehyde reaction have been noted in the chapter concerning modified starches, particularly the utilization of the products of the early stages of the reaction. Starches of rather high viscosity, which find some application as sizes in paper manufacture (60) and in certain types of adhesives, are produced. The extended action of aldehydes on the non-cereal

⁶ A comparable structure has been proposed by Maxwell (59) to account for the high viscosity of starch products that have been reacted with bifunctional reagents such as dibasic acids and epichlorohydrin.

starches, such as potato and tapioca, to induce them to form firm, transparent gels has also been mentioned. The action is rather unique.

Additional applications of the reaction in its more extended phase are noted in the patent literature. Classen (61, 62) has patented the use of his starch-formaldehyde compound, mentioned above, as an antiseptic surgical dressing. Blumer (63) and others described (64-66) the manufacture of both water-soluble and water-insoluble adhesives and plastics. The more important applications at present are apparently those, however, in which other condensing agents are also present. Phenols, ammonia, and urea, respectively, are examples of the additional reagent used. In these cases, no doubt, there is a competitive reaction between the starch and the other condensing agent. The extent to which the starch enters into the reaction is unknown and, indeed, it is questionable whether the starch enters into the reaction at all, in some cases. Native, acid-modified, and thin boiling starch and dextrans have been used in these types of reactions or modifications.

Loetscher (67) produced a water-soluble resin by adding 5 parts of 5% NaOH to 100 parts of water containing 100 parts of carbohydrate, followed by the addition of 100 parts of phenol and 100 parts of 40% formaldehyde. The mass is heated to 200-225° F. and held at this level until it becomes clear and viscous, but the heating is stopped just before the resin becomes water-insoluble. Berquist (64) treated thin boiling starch with formaldehyde and an ammonium compound, which was then further hydrolyzed to produce a clear, viscous adhesive, as follows: A mixture of 190 g. of corn starch, 225 cc. of water, 0.3 g. of NH_4Cl , 0.65 g. of formaldehyde (40%), and 0.5 cc. of HCl (23° Bé.) is stirred for 30 min. and filtered. The product is dried, first at a low temperature and then at increasing temperatures until 170° F. is reached. The starch product is held at this temperature until the desired viscosity is attained. Leuck (65) dextrinized starch in the presence of ammonia, and then treated the product with formaldehyde and acid to produce a water-resistant adhesive.

The present trend to conserve wood in the manufacture of containers for food products, general merchandise, and munitions by use of water-resistant, paper board, fabricated from laminated paper, is facilitated by the use of water-resistant sizes and adhesives made from starch products, urea, and formaldehyde (68). Thick starches and dextrans or combinations of the two are heated in the presence of a urea and formaldehyde, or a product which liberates formaldehyde, until the starch product is dispersed in the water present. Either an ammonium salt or a weak acid, or a combination of both, may be added to catalyze the condensation which follows after the size or adhesive is applied. With applied heat, or after an aging at lower temperatures, the product develops insolubility to water. Several formulae are currently used which produce surprisingly water-resistant, laminated paper board. Taft (68) has discussed some of the properties of urea-formaldehyde resins and their application as adhesives in paper manufacture. The application or use of starch products in this type of formulation will be discussed further in Chapter XXII on adhesives.

3. Hydrogenation of Starch. Of the common reactions (oxidation, etherification, acetylation, etc.) usually applied to polymeric materials, hydrogenation has been the least studied. This is particularly true in the case of starch. Specific references to the hydrogenation of starch are extremely rare in the chemical and patent literature. Generally, a report is concerned with the hydrogenation of a simple carbohydrate, such as glucose, and starch is included as an additional carbohydrate material which, presumably, should yield the same end-products.

Recently, Yoshikawa (69) and Yoshikawa and Hanai (70) made a study of the hydrogenation of starch and other carbohydrate substances with nickel and nickel-iron catalysts. Under certain conditions hexitols were obtained; under more drastic conditions, the products were glycerol and propylene glycol. Thus, for example, 10 g. of potato starch, suspended in 50 cc. of water, were hydrogenated with a Ni-Co-Fe catalyst at 150° C. under 100 atmospheres pressure for 1 hr., whereupon the starch paste was liquefied. A second hydrogenation stage at 220° C. was then carried out for 3 hrs. to yield *D*-mannitol and *D*-sorbitol. Similar results were obtained with corn, wheat, and rice starch.

TABLE XXIX

Products Obtained from Carbohydrate Materials (Per Cent of Original Material) by Treating with Hydrogen at 300 Atmospheres over CuCrO at 250° C. for 2 to 3 Hrs.

Compound	CH ₂ OH	C ₂ H ₄ OH	H ₂ O	C ₆ H ₅ (OH) ₂	Hexitols	Residue	Total
Glucose	3.3	5.5	14	50	10.5	9.5	92.8
Sorbitol	3.3	7.8	10	51	15	Trace	87
Mannitol	2.2	8.3	15	50	15	2.8	93.3
Maltose	2.8	7.8	18	33	12	17.5	91
Methyl α - <i>D</i> -glucoside ..	4.5	4.5	16	15	20	2.2	62.2

When the liquefied product was hydrogenated at 220° C. for the 1st hr. and then at 260° C. for 4 hrs., the main products were propylene glycol and glycerol. Detailed descriptions are given for the preparation of the several catalysts used. The yields of products, however, are not reported. Similarly, processes described in the patent literature (71) are very vague with respect to statements concerning the yields of glycerol and propylene glycol from starch. Other catalysts which are claimed to effect the type of hydrogenation outlined include those containing copper, silver, gold, or tungstic acid (71).

The fact that the yields of glycerol and of propylene glycol are not reported and that very little research on the hydrogenation of starch has been attempted is understandable if one considers the hydrogenation of starch in a hypothetical manner, using the present knowledge of hydrogenation and hydrogenolysis reactions as a basis for discussion. The noteworthy work of Adkins and co-workers (72) on the hydrogenation of organic compounds with copper chromite and Raney's nickel catalysts makes such an attempt possible.

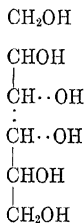
It has been found by Adkins that glucose, mannitol, and sorbitol yield the same products in substantially the same amounts upon hydrogenation with copper

chromite at 250° C. for 2 hrs. (73) (Table XXIX). Since it is known that the carbonyl group is readily reduced to a hydroxyl group at relatively low temperatures (glucose yields sorbitol in a 97% yield at 160° C.) and since the same products are obtained from glucose and sorbitol at 250° C., it may safely be assumed that sorbitol is an intermediate in the hydrogenation of glucose at high temperature. Therefore, should any glucose be formed during the hydrogenation of starch, the final products should be similar to those obtained from sorbitol. However, except when the hydrogen ion activity is not controlled, it is more likely that cleavage of the starch molecules takes place by means of hydrogenolysis, rather than hydrolysis. In such an event it would be desirable to know the conditions required to cleave acetal linkages. Unfortunately data on this subject are too incomplete to permit of an accurate comparison with starch. The available evidence, however, indicates that 175° C. represents a minimum temperature for the hydrogenolysis of such a linkage with catalysts containing nickel, copper, chromium, or cobalt (74).

The data accumulated by Adkins on the hydrogenation of maltose and methyl α -D-glucoside are important (Table XXIX). It is quite apparent that the introduction of a glycosidic linkage has a pronounced effect on the nature of the hydrogenation products, the most noticeable change occurring in the yields of propanediol-1,2 (propylene glycol) and the high boiling residue. It would be expected that an increase in the number of glucose residues in a series of polysaccharides would result in a decrease in the yield of propylene glycol and an increase in the yield of high boiling residue. This result has been observed in the hydrogenation of cellulose, when a dioxane suspension, CuCrO catalyst at 250° C., and 300 atmospheres of hydrogen pressure for 5 hrs. were used, and the yield of high boiling residue varied from 25 to 30% of the original material (75).

It is noteworthy that no glycerol was obtained in the experiments reported by Adkins. Although it is not inconceivable that glycerol can be isolated from such reaction mixtures, it is improbable that this material would be obtained in appreciable yields, owing to the susceptibility of 1,3-glycols to hydrogenolysis. Glycerol, for example, is converted almost quantitatively to propylene glycol by CuCrO at 250° C. in 4 hrs. (76).

Hydrogenolysis of carbon-carbon linkages is also common with glycols, especially those containing hydroxyl groups in the 1,3 positions. Adkins summarizes the hydrogenolysis of simple sugars according to the accompanying scheme, the probable weak bonds being indicated by dots in the formula for sorbitol.



Thus it would appear that only small quantities of sorbitol, mannitol, or glycerol are obtained as hydrogenation products of starch with catalysts of the series, Ni, Fe, Cu, Cr, and Co. These quantities are too small to be of technical importance. The main products of starch hydrogenations are methanol, ethanol, water, propanediol-1,2, and high boiling compounds of unknown composition. Utilization of this reaction with starch may not, therefore, be confined to its use as a primary modification of starch. Rather it would seem likely that its technical value, if any, would be as a secondary reaction to be applied to starch, premodified by other means. The possibilities of a preliminary or simultaneous hydrolysis of the starch to increase the yields of utilizable substances of low molecular weight, such as the glycols, should be evident from the foregoing discussion. Other preliminary modifications of starch suggest themselves; *e.g.*, oxidation and dextrinization. When controlled, oxidation following hydrogenation should lead to the production of unusual glycols or hydroxy acids, depending on the type and extent of the preoxidation. The use of hydrogenation to convert undesirable carbonyl groups to non-reducing groups in torrefaction dextrins has been patented (77).

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SECTION FIVE

USES

The uses for starch are as numerous and varied as are the sources from which it is derived. The task of cataloguing and detailing the properties of the innumerable botanical types of starch is no more difficult than tracing the utilization, or application of the product, as it affects each of us throughout every hour of our daily life. Starch may be used in the manufacture of soaps and other cleansers. It is used in the manufacture of the clothes we wear and in the laundering which keeps them serviceable. Starch, in some modification, or as a derivative, is an important constituent in the morning breakfast. It has been used at the foundry in the production of the vehicle which transports us to our work and returns us to our homes. Starch has no doubt entered into many of the manufactured structural elements or into the building operations used to make the place in which we work and the home in which we live. It is used in the manufacture of paper, such as writing paper, and paper products, such as boxes, containers, and labels, without which modern commerce would be at a serious disadvantage. Starch may be used in the manufacture of the furniture and rugs which equip our offices and homes. It is used in the preparation of soups, sauces, meat products, sirups, puddings, cakes, and a host of other items which make up the daily menu. Starch, or a derivative, is used in making candy, ice cream, ices, and some of our more popular beverages, both alcoholic and non-alcoholic. The largest single application for the production of a product for human consumption is in the manufacture of beer. Starch is used in the manufacture of such textiles as towels, table cloths, and bed linen, which we have considered indispensable to modern life.

In addition to those mentioned, starch has many other industrial applications as diverse in character, as for example, the refining of ores, such as aluminum, the manufacture of rubber goods, and the use in drilling oil wells.

A complete discussion of all of the many applications of starch products must be left to a more comprehensive work on this particular phase of the subject. The following chapters present an outline of those which have been traditionally considered as the most important. These happen to represent, as classes, the most important applications in respect to the volume of starch used. They are the manufacture of paper, textiles, and adhesives and the fermentation and food industries.

CHAPTER XVIII

USE OF STARCH IN PAPER MANUFACTURE

1. Introduction. Judged by the volume of starch consumed, the paper industry is one of the most important, non-food outlets for starch and starch products. Starch is used in various sizing operations during the manufacture of paper sheets and paper boards, and also in fabricating these into various containers and cardboard articles. In the latter cases, the starch product is used more as an adhesive, as in joining two or more sheets to form a laminated or corrugated board, to manufacture various kinds of boxes, bags, envelopes, cartons, and other containers. Although these manufacturing operations are an integral part of the paper industry, nevertheless, the use of starch products in these instances will be dealt with under the general subject of adhesives. This division in subject matter is purely arbitrary, inasmuch as an important function of starch in sizing is to create a bond between units in the paper; that is, act in the nature of an adhesive. However, this present section will be devoted to discussing the function and use of starch products in the various operations employed to form and finish the sheet of paper.

In the manufacture of paper, starch is said to be used as a size. Sizing is accomplished in one or more of three phases of the manufacture. It is added to the pulp, at the beaters, before the sheet is formed; it is applied as a so called surface size, as for example, tub sizing; and, lastly, certain papers, such as high grade magazine papers, are sized with coatings in which clay or other pigment is usually incorporated to produce a sheet which will have better characteristics for printing. Each type of sizing will be dealt with separately.

Paper is made from a pulp consisting essentially of cellulose fibers obtained from various sources. For the better grades of writing paper, in which durability is desired in respect to fold and tear, a part of the pulp is of cotton origin. Old rags are bleached and disintegrated to furnish a proportion of long fiber. Intermediate grades of white paper are made essentially by chipping certain varieties of wood, digesting the chips in caustic soda, sulfite, or other hydrolytic agents to remove lignin and other undesirable constituents, and, finally, bleaching. In the manufacture of wrapping and bag paper, bleaching is usually omitted. The lowest grades of paper contain substantial quantities of ground wood which, as might be expected, contains mostly short fiber. Certain paper products, such as paper board of the corrugated type or wall board used in building insulation, may contain substantial amounts of reclaimed paper, straw fiber, sugar cane fiber, or fiber from other sources.

2. Beater Sizing. After the crude pulp has been digested, washed, and bleached, if a light colored sheet is to be made, it may be dried and stored for future use or it may be passed on immediately to the beaters. Here the pulp is disintegrated further and dispersed into a relatively large amount of water by

mechanical action. One object is to separate individual fibers so as to form a uniform sheet (or web, as it is called) when the pulp is collected on a screen filter, referred to as the "wire." Another object is to "hydrate" the fiber to the desired degree. Beating operations vary depending on the pulp stock used and the type of paper being made. However, certain general observations can be made, the understanding of which is essential properly to evaluate the merits of added sizes: (a) The strength developed in the paper sheet depends on the extent of beating. Normally, the strength slowly increases with the amount of beating up to a maximum. After many hours of beating a pulp suitable for making paper referred to as "glassine" is finally obtained. (b) The ability of the pulp to retain water when passed over the "wire" (as noted by allowing the suspension of pulp to settle in a tall glass cylinder) is increased to a maximum, depending on the extent of beating. This water-retaining ability is referred to as hydration, although it is doubtful that a cellulose hydrate is formed. It might be concluded that the strength developed in the paper depends on the extent to which the pulp is hydrated. (c) It follows that in the use of undried pulp in the beaters, that is, in a process in which manufacturing operations are continuous, less beating action is required to secure optimal results than in a process in which a dry pulp is used to supply the beaters. (d) Different types of pulp stock may require different degrees of beating to bring them to their respective optimal states of hydration. This difference is due to the source of the fiber, the extent of chemical digestion, and other variables in pulp-making. Other generalities will be given after a discussion of the added size.

The function of a starch size added at the beaters is primarily, as intimated above, to increase the strength of the sheet. Depending on the nature of the sheet to be made, less important functions are those to aid in making the sheet more impervious, to lay fuzz, to impart a crackle to the finished sheet, and to aid in making the sheet more resistant to such wearing actions as scuffing and folding.

It will be seen that a suitable sizing agent is one which is colloiddally dispersed sufficiently to enable it to form a bond between the individual pulp fibers, and yet is not degraded to the extent that its adhesive strength is low or that it has become so water-soluble or dispersed as not to be retained by the fibers but rather will pass out with the water phase when the pulp is filtered on the "wire." Considerable effort has been spent in research directed toward the attainment of an optimal balance between these factors. As a consequence, starch has been used for sizing in the beater in a great variety of forms and states of pretreatment.

Raw, or ungelatinized, native starch has been added at the beaters, either owing to a lack of appreciation of the above considerations or to the possible supposition that if the starch were gelatinized, or solubilized, it would more readily pass into solution in the water phase and as a consequence less starch would be retained by the pulp. When raw starch is used, it is expected that sufficient heat and water will be available to gelatinize the added raw starch, which is mechanically held by the paper pulp, when the newly formed, wet sheet

is subsequently passed over heated rolls to dry the paper. Raw starch has so little adsorptive affinity for most types of paper fibers, however, that of the 1 to 3% starch normally added to pulp in beater sizing only about 30% of this starch is retained by the fiber. The amount that is retained, if examined in the finished sheet after being stained with iodine, will be seen to be very poorly dispersed (1). That is, the heat and moisture available at the drying rolls are not sufficient to gelatinize completely most raw starches, *e.g.* corn starch, and hence only a fraction of the starch retained becomes effective in creating a bond between the paper fibers.

To secure better efficiency in the use of an ungelatinized starch, it may be modified in manufacture to reduce its gelatinization temperature. For example, Casey (2) has shown the increased effect on the Mullen test for tensile strength, fold, and ink resistance tests secured (over unmodified starch) by oxidizing the starch before adding it to the beaters. The extent to which the starch is modified by oxidation, as judged by paste viscosity tests, does not significantly alter its efficiency, except in ink resistance. This result is in contrast to some of the other types of starch modification such as acid hydrolysis.

The next logical step in the efficient utilization of the starch is to cook the starch before adding it to the beaters. This secures greater colloidal dispersion with the result that, instead of a granule of starch bonding two fibers at only one point, the adsorbed or absorbed starch paste will be spread over a greater area and hence bonding between fibers will be secured (by the one original granule, for example) at many points. The underlying difficulty in this procedure is that, whereas raw starch is surprisingly resistant to mechanical treatment (such as to the disintegration applied in the beaters but more particularly in the Jordan disintegrators which frequently are used after the beaters), gelatinized or partially gelatinized starches, particularly the non-cereal starches, are very unstable to the action of physical forces. The result is that the degree of colloidal dispersibility of the gelatinized starch does not remain constant, but rather the starch continues to disintegrate and a large part of the starch is broken down to such an extent that it is lost at the "wire" (3). The extent to which starch should be cooked becomes, therefore, a matter of judgment and is conditioned by the type of raw starch used, by the differences between individual batches of any particular starch, by the pH of the available water, by the variation in the operation of the beaters and Jordan disintegrators, and so forth.

Methods have been proposed to circumvent the above difficulties. One method is to stabilize the cooked starch. Rowland (3), for example, cooked raw starch in the presence of formaldehyde and acetic acid and claimed that he secured the desired toughening action on the granules. A modification of the procedure is to pretreat the starch with formaldehyde and then disperse it by cooking it with sodium hydroxide before it is added to the beaters. This dispersed starch is precipitated on the fibers by the addition of acidic material, *e.g.* alum.

In respect to the latter procedure, other proposals have been made to secure better retention of rather highly dispersed starch on the paper fibers. Precipi-

tants such as alumina have been recommended for this purpose (4). Kesler and Black (5) proposed that fatty material, *e.g.* fatty acids or soaps, be added, since they believed that a complex formation occurred with the dispersed starch. The complex is then precipitated on the fibers by the addition of metallic ions to form an insoluble metallic soap-starch precipitate. The fundamentals involved in the use of such precipitants on starch have been discussed by Heald (6).

The value of insolubilized starches, that is their bonding strength for cementing the various fibers together, is not very well known, as little has been reported concerning the relative increase in bursting, fold, tear, and scuff strength, permeability, etc., for a given addition of size.

It has been suggested that better efficiency will be obtained if the starch is chemically modified before or after the cooking process. For example, Cobb and coworkers (7) state that starch behaves like a highly hydrated cellulose. Very viscous starches, carrying relatively large amounts of water, when dried may induce an undue contraction in the sheet. If present in the amount required to bond all the fibers, the viscous starch may contract so much that air cells develop on drying and the starch films would not be continuous. These workers demonstrated that modification of tapioca starch, by enzymic action during the period of cooking, increases the drainage rate at the "wire," and improves the results with the Mullen and "pick" tests.

An additional objection to precooking the starch, raised by some of the larger mills, is that another operation is introduced into paper manufacture.

Starch millers and processors, working with the paper industry, have, therefore, sought to pretreat the starch and have experimented with methods to precook it so that the optimal benefits will be secured by adding to the beaters the dry products as supplied. The pretreated and precooked starches are prepared in the final dry form by passing the starch over heated rolls, by vacuum drying with the help of agitators, by spray drying, etc. The most commonly used product is dried over hot rolls and is sold under the trade name "Amijel."

As reference to chapters on the physical and chemical properties of the starches will show, gelatinization of the starch induces a physical lability in the linear components of the starch, commonly called amylose. After the starch is solubilized in hot water, the amylose may irreversibly precipitate on aging and cooling. This phenomenon is often referred to as retrogradation. Once precipitated, amylose is not redispersed unless subjected to temperatures higher than 212° F. or to the action of alkali or some other chemical reagent. Hence it is impossible to obtain the equivalent of a freshly cooked starch paste with many of the pregelatinized products simply by the addition of water. A granular-appearing paste is obtained which, even after an extensive disintegration treatment in the beaters, is not sufficiently broken down to produce the desired dispersion. Although fair retention may be obtained with such a product, dispersion is poor, as reference to the data and Figs. 94 and 95 will show. The net result is poor over-all efficiency for such a product. Fig. 94 shows an iodine-stained sheet made by adding 2% of a roll-dried corn starch product to the pulp

at the beaters. Obviously the extent to which a roll-dried product is ground after leaving the drying rolls will influence its dispersibility. Overgrinding may, however, reduce its retention in the paper fiber at the "wire." The particle size of the product becomes an important factor, consequently, in respect to its efficiency for beater sizing.

As mentioned before, alkalis and alkaline reagents tend to redisperse retrograded starch. Hence borax is incorporated in some pregelatinized starch products to facilitate redispersion. The borax serves an additional purpose in that it also acts as a wetting agent when the product is remoistened. This function will be mentioned again later.

Some starches retrograde less under the same conditions than others. Potato and tapioca starches show less inherent tendency to retrograde than certain cereal starches, as for example corn starch. Pregelatinized products made from the former starches, particularly if the product contains a small amount of dispersing agent, may be stirred up and beaten into a paste which approximates a freshly cooked potato or tapioca starch paste in colloidal properties. Properly used, such products have good dispersion in the beaters, good retention, and hence a relatively high efficiency as beater sizes. Disadvantages to be noted for these non-cereal products include the following. (a) Their dispersed pastes are relatively unstable to mechanical treatment. (b) They are made largely from imported starches. For this reason they are at times expensive or their supply is uncertain. Native potato starch is one of the most expensive domestic starches. (c) They wet rather poorly and tend to ball up when stirred into water. The particles take up water at such a rate that some form a gummy mucilage which then encases or surrounds many other dry starch particles. These larger aggregates beat out with difficulty.

In addition to having the inherent property of retrograding less, potato and tapioca starches are more viscous than corn starch. This latter property may be of aid in obtaining greater size retention for a satisfactory degree of dispersion.

One of the oldest and most commonly used methods for the industrial modification of corn starch to eliminate its retrograding tendencies is by oxidation, as for example with sodium hypochlorite. Simple oxidation for the purpose of minimizing this undesirable characteristic in a starch which is to be roll-dried, however, also results in such a physical degradation of the starch that the resulting product, when redispersed, is too low in viscosity for high efficiency as a beater size.

A method for pretreating a starch such as corn starch with chlorine which reduces the retrograding tendencies to such an extent that a roll-dried product may be made which redisperses in the beaters to a very satisfactory degree has been proposed by Kerr (8). At the same time so little degradation of the starch units accompanies this pretreatment that retention is not impaired. The result is an over-all efficiency for the product which compares very favorably with those made from potato and tapioca starches. The addition of borax to such a pretreated corn starch before it is passed over heated rolls, permits the gelatinized,

starches and dextrans, are rarely employed. In order to obtain paper surfaces more suitable for some uses or to obtain special effects in finishing, other ingredients may be added to the starch size; for example, wax emulsions. Some of the special starches for calender sizing are prepared by the starch manufacturer by the inclusion of suitable waxes or similar ingredients.

4. Tub Sizing. The fundamentals of starch chemistry as applied to paper sizing have been discussed by Kerr (9) with particular reference to tub sizing. Since in this application the preformed sheet is immersed in a dilute size solution of low viscosity, it is desirable that the starch product be pretreated to reduce its paste body to the level at which the size will penetrate the sheet to the desired extent and will add dry substance to the finished sheet within the limits of weight desired. Reduction in paste viscosity can be obtained in several ways. The more frequently used methods are by the use of acids, oxidizing agents, conversion by enzymes, and dextrinization by heat. For the purpose of tub sizing paper, it is obvious that a method for modification should be selected which will either tend permanently to solubilize the amylose or will tend to degrade it preferentially, leaving the amylopectin in a high state or degree of polymerization. Incorrectly modified starches, in particular corn starch, are very readily recognized by the poor results obtained in practice from such poorly modified products. The viscosity of the size will increase on standing or it will increase during the period of time the size is used. An increase in the viscosity of the size may result in less penetration and an increased weight of starch applied to the paper, usually on or near the surface of the sheet. Tub sizes are normally made by diluting starch pastes to a relatively low concentration of solids. The amylose, if insufficiently modified, becomes unstable in colloidal solution when the paste is diluted and it tends to separate as an insoluble phase. From these sizes the liquid phase is preferentially absorbed by the dry paper sheet and the insoluble material is continuously returned to the size box by the action of the squeezer rolls through which the paper sheet passes after leaving the size box. This effect increases the consistency of the size. Eventually, the insoluble material tends to cohere and lumps become mechanically enmeshed in the paper fiber. Very uneven sizing results, giving the paper an unattractive appearance.

Animal glue has been and is being used to tub size paper. Some paper manufacturers, especially those making certain types of white writing paper, prefer to use mixtures of glue and a starch product in order to secure the beneficial results of each. Glue or certain constituents of a glue size are not compatible with solutions of undegraded amylose. Even in dilute solutions, the stability of the amylose is reduced in such mixtures by the presence of the glue size. When some glue is employed, it becomes increasingly important, therefore, that the amylose component of the starch be degraded or solubilized to such an extent that it will not retrograde.

Oxidized starches have been used to good advantage in tub sizing. The stability of oxidized corn starches is well known. However, their price is rela-

tively high compared to other modified starches and to some of the other sizing agents available.

Very good results have been obtained in recent years when a properly prepared torrefaction dextrin was used. The proportion of linear chained polymers in some of these dextrins is small, as judged by butanol precipitation (9). The results obtained with dextrins of this type are in harmony with the theories advanced by Brimhall (14) that in the process of dextrinization by heat it is possible to join the linear polymers by new glucoside linkages to form branched configurations.

Principally for reasons of economy in recent years there has been a most remarkable increase in the amount of starch used by the paper manufacturer for tub sizing that has been converted with enzymes. This modification and application of starch is discussed elsewhere.² The success of the method in this instance depends upon selecting the proper conditions to direct the enzymic action so that in the liquefaction of the starch to the desired viscosity, the amylose will not be left in a state in which it will interfere with the paper-sizing operations or results. In this connection the work reported by Kerr, Meisel, and Schink (15) should be noted. Technologists of the paper, enzyme, and starch-milling³ industries have cooperated in this development with the result that the paper manufacturer already has at his disposal methods for making acceptable tub sizes by the use of enzymes and of native starch. Judging by the rate at which these developments have been observed in practice, it may be concluded that ideal tub sizes may soon be produced by such means.

5. Surface Sizing: Clay Coatings. Certain types of paper are surface-coated with clays or similar fillers. The size may also contain colored pigments, water-proofing agents, or other substances to give the desired weight, color, and finish to the sheet. For example, the better grade of magazine or periodical paper is surface-coated with a clay mixture which, after being dried and ironed over calender rolls, takes on a glossy surface which is relatively impervious to printers' ink. Naturally, better printing is possible and sharper photographic reproductions are obtainable. Many other diverse types of coatings besides clay coatings could be listed. The art of paper coating is very complex indeed, and it is beyond the scope of this chapter to discuss all types. Only those which involve the largest outlet for starch products will be treated. Coating may be applied to paper by any one of several methods, and the discussion is divided according to the method of applying the coating, since the process which is employed in a large measure determines the characteristics required in the coating.

The older methods involve passing the predried paper sheet between applicator rolls which apply a given amount of size to the paper. The paper then passes through a series of brushes which spread the coating evenly over the paper surface. The paper is dried by passage through a heated chamber and is calendered. Both sides of the paper are usually coated in the same operation. In

² See Chapter XVI.

³ See, for example, the recent report of Gillan (16).

one modification of the above process highly polished metal rolls are used instead of brushes to spread the coating. Another modification is the use of a fine jet of air at high velocity to spread the coating and to remove the excess which then runs back to the size box. Although a relatively high speed may be maintained on such machines, difficulties are encountered when attempts are made to coat both sides of the paper simultaneously. The above types of coatings, as a class, are referred to as "brush coatings" and the characteristics required in the coating are very nearly the same for each method mentioned. The principal determining factor is that operating conditions for this type of work usually require that the coating contain not more than 55 to 60% of water. The use of more water frequently results in a failure to put the required weight of clay on the paper, and in addition requires more heat, or drying time, for drying the coated paper than when smaller amounts of water are used.

However, clay mixed with an equal weight of water is an unworkable mud. Even if it were thinned to a usable consistency, it would not adhere to the paper fibers when the paper is dried. Hence, wetting agents, protective colloids, and adhesives are added to the clay to secure the proper colloidal characteristics. Casein, cooked in alkaline media, is very nearly an ideal colloid for this purpose. The addition of 5 to 10% of casein reduces a clay mud to a mobile liquid which may be evenly brushed over the surface of the paper. The casein has, furthermore, a high adhesive strength in binding the clay to the paper fiber. It is, however, even in normal times, a relatively expensive product to use in the manufacture of paper. Starches have therefore been modified to replace casein. The advantages to be listed for the use of starch as compared to the use of casein are that the starch is free from odor, is non-nitrogenous, and possesses fewer inherent foaming tendencies. Being non-nitrogenous, it is less susceptible to decay and, hence, the starch-coated papers possess greater permanence. Excessive foaming is to be avoided in coatings, since froth tends to create a pitted surface on the sheet. To secure the desired adhesive strength, more starch is required, pound for pound, than casein. For this reason and also in order to develop greater protective colloidal properties, the raw starch is modified. This modification includes a reduction in paste viscosity so that the final starch-clay mixture will not be thickened because of the added starch. The most common modification of starch which accomplishes the results described is chlorination. Highly chlorinated starches are used in a ratio of about 15% starch to clay. The resulting coated sheets are similar in many respects to those sized with casein-clay coatings. The starch-clay coating does not possess quite the adhesive strength or the water resistance of the casein-clay coating. Water-resistant surfaces are desired for certain papers. Naturally, better bonding between clay and paper fiber results by adding a higher ratio of starch to clay. Two undesirable effects are noted as a result of this practice: a duller sheet is obtained, that is there is a loss of gloss, and, secondly, the viscosity and the plasticity of the coating are increased with the result that it becomes more difficult to spread the coating during the paper-sizing operation. To correct

this effect either more water may be added to the size, which addition slows the production rate in the drying operation, or a starch that is more highly modified and of thinner viscosity may be employed. Experience has shown, however, that if starch is modified beyond a certain point degradation reactions then predominate to the extent that the adhesive strength and the protective colloidal effect of the starch are reduced at a more rapid rate than the viscosity and plasticity of the starch are reduced.

No doubt much of the unfavorable reputation established for starch in paper coating can be traced to improper application due to a lack of appreciation of the characteristics of starch, outlined above. Another factor contributing to the improper use of starch is that a sol, prepared by cooking a highly chlorinated starch, is a relatively labile, colloidal system. Excessive cooking of the starch, or excessive heat at any point in the operations which follow, tends to degrade or destroy the colloidal properties of the starch. Excessive mechanical action, such as occurs in mixing the clay, starch, and water as well as the agitation of the size in the circulation pumps and at the coating machines, tends to thin and weaken the starch. The working characteristics of a batch of clay and starch may, therefore, tend to change during the period of its use. When poor results start to develop, as noted first by a decrease in the viscosity of the size, then by poor binding action of the starch in holding the clay to the paper fiber, the natural tendency of the operator is to add more starch to the unused portion of the batch of size. An increase in plasticity is often noted as a result of such corrective procedure by the development of streaks in the sized paper due to poor brushing. If more water is added to secure smoother brushing, the size not only requires more time or heat for drying but also becomes weak in adhesive strength.

In normal periods, the difference in price between the so called chlorinated starches, or gums, and casein hardly compensates for the variation in the grade of coated paper produced. This variation results from a lack of appreciation of the behavior of chlorinated starches, a failure to exercise proper control in their use, or a combination of these factors. In recent years, starch chemists, working with paper chemists, have devoted considerable effort to finding improved methods for modifying starch and for applying the starch product. It should be noted before passing on to these developments that the starch chemist has, in the meantime, made considerable improvement in the colloidal stability of chlorinated starch. Several brands are now on the market which are decidedly improved compared to those formerly marketed.

With chlorinated corn starch as a reference or standard for the comparison of starch products in clay coatings, other means of modifying the starch will be discussed, and the resulting products will be compared with the standard in an attempt to develop certain general principles.

If corn starch is modified by an acid treatment to such a degree that the hot paste viscosity of the product is similar to that of the chlorinated starch referred to above, and a clay coating is made with this acid-modified starch, it will be

observed that the coating is more plastic in nature and that the adhesive bond formed between clay and fiber is weaker than in a similarly prepared coating in which chlorinated starch is used. To explain the plasticity of the coating, it may be noted that the acid-converted starch tends to gel or retrograde more after cooking and cooling than the chlorinated starch and that its protective colloid effect on the clay is less. The action of acid in reducing the paste viscosity of a starch is accomplished primarily by a scission of glucoside bonds between the glucose units which make up the various starch molecules. These ruptures weaken the physical structure of the starch granule and thereby reduce its paste viscosity. As the action is extended, there is a decided decrease in the average molecular magnitude of the constituents of the granule and there is a parallel loss in the adhesive strength of the modified starch. It is to be noted that in the intermediate stages of the hydrolysis the percentage weight of starch precipitable by butanol increases to a value above that for untreated starch. Hence it must be assumed that before a material degradation in the linear chain components of starch is accomplished by the acid, the branched components suffer a decided decrease in their molecular magnitude (breaking apart in fragments) to form additional quantities of linear polymers. Chlorination, on the other hand, reduces the paste viscosity of a starch without reducing (actually increasing) the colloidal stability and the protective colloid effect of the starch, both of which properties are usually associated with the amylopectin fraction. The percentage weight of starch precipitable by butanol gradually decreases to zero during an oxidation of starch with chlorine.

From the foregoing discussion it might be presumed that the ideal starch product to use in preparing a clay coating would be made by destroying the granule structure by extensive gelatinization, either by cooking at high temperature or by solution in alkali and thereafter freeing the amylopectin from the amylose. This would seem to follow from the result that the isolated amylose fraction possesses a viscosity that increases abnormally with an increase in concentration or with a decrease in the temperature of solution; also, that its solutions show exaggerated tendencies to gel and that it possesses, if any, a negative protective colloid effect on clay suspensions. The author has performed such a separation using butanol to precipitate the linear polymers from the amylopectin and has attempted to prepare coatings from both of the separate fractions.⁴ The results of this experiment disclose that the amylopectin is too viscous to be used as such in clay coatings if the usual ratio of about 15% of starch product to clay is employed. The use of a reduced concentration of amylopectin in order to obtain a clay coating of workable viscosity results in a coating on paper with an adhesive strength below that desired; that is, the clay is not sufficiently bound to the paper fiber after the coating is applied and dried. Some degradation of the amylopectin fraction is therefore required. As might be anticipated, the complementary fraction of the starch, the amylose, could be

⁴ Unpublished results of Kerr and Schink.

studied in clay coatings only when a low concentration of the product was used and when temperatures above 70° C. were employed for application of the coatings. With these experimental conditions, the coating could be evaluated and it was found that the amylose-clay coating proved to have a higher adhesive strength than amylopectin at a like concentration. Either an increase in the amylose concentration to obtain a coated paper suitable for printing or an attempt to apply the coating to paper at a temperature normally used in practice results in an unworkable plastic mass of clay which very soon gels. Thus, although native amylose possesses better adhesive strength in this application than native amylopectin, the latter has all of the other colloidal characteristics desirable in an adhesive-carrier for clays except that in its native state its viscosity is too high. Since some degradation or modification of the amylopectin fraction is desirable, the possibility of starting with whole starch and designing a modification which will preferentially degrade the linear chains at a greater rate, or which will alter the colloidal characteristics of the linear chains in a desirable fashion during the modification, is worth considering.

Possibly when starch is modified by oxidation to a usable viscosity⁵ for clay coatings, the amylose is preferentially degraded or modified, as has already been pointed out by Kerr (9) from experiments made by Peckham and Newton. Chlorinated starches are, however, relatively expensive. Moreover, their use in practice requires careful control, since they are colloiddally less stable to heat, mechanical agitation, pump pressure, and higher pH when cooked into a paste than corn starch modified by some of the other methods available.

The use of enzymes to modify the starch for use in clay coatings has been studied. Enzymes are selective in their action in respect to the chemical configuration of the substrate. It is known that the linear components of starch are readily hydrolyzed to maltose by diastase, particularly by the β -amylase constituent of commercial diastases. The branched configurations present barriers to an extensive degradation by the saccharogenic factor, β -amylase. It seems possible that by selecting the proper combination of amylases and by adjusting conditions for conversion it would be possible preferentially to modify the less desirable amylose constituent. At the same time it seems possible to eliminate the effect of the granule structure of the starch on the paste viscosity and to reduce the viscosity of the resulting colloidal solution of amylopectin to the desired value. Several problems are involved in the use of this method, a solution for which is not readily apparent. β -Amylase speedily and completely degrades the linear glucopyranose chains which are available by producing sugars such as maltose. Since sugars have low adhesive strength and poor colloidal properties, that part of the amylose split by the enzyme to sugars is a

⁵ The work of Diehm (17) suggests that the highest clay-binding power of a starch product results when the greatest reduction in viscosity is obtained for least reduction in molecular magnitude. Considering the abnormal viscosity effects produced by amylose and that the latter contributes little to the average D.P. value for starch, this view can be harmonized with the one presented above.

loss as far as coating properties are concerned. Furthermore, not all of the linear polymers in starch are readily available to the enzyme. Some part of this fraction is apparently in a physical state which makes it almost invulnerable to attack by the enzyme.

The use of commercial enzyme preparations which contain a predominant amount of α -amylase may lead to the production from amylose of dextrinous bodies which will retain a sufficient length of chain to contribute to the adhesive strength of the converted starch. Nevertheless, it is likely that a share of the amylopectin also will be reduced materially in molecular size in the presence of this enzyme by the more random splitting of glucosidic linkages. That is, the hydrolysis with α -amylase appears to lead to results similar to those obtained by acid hydrolysis.

To overcome one of the major objections to enzymic modifications given above, Kerr and coworkers (15) have suggested that the starch processor might pretreat the native starch with enzymes, using such conditions that a considerable portion of the linear components of starch is removed as sugars of low molecular weight. These sugars can be removed by washing and recovered to be sold for some suitable use. The residual starch can be additionally modified with enzyme by the paper mill technologist to produce a superior starch and clay-coating mixture.

Considerable research by paper technologists is in progress on methods for the preparation of superior coatings in which is used starch that has been modified by a single treatment with enzymes at the paper mill. In general, sizes made from enzyme-converted starches, such as corn starch, show a measurably more plastic character than similar sizes prepared from chlorinated starch. However, a very significant contribution has been made by the paper chemist in recent years which not only permits the use in clay coatings of such enzyme modifications but of other types of modified starches, the use of which will be discussed.

It has been known that a part of the plasticity of a clay coating is due to the presence of the clay itself. The relative proportion of the plasticity of the coating for which the clay is responsible has not been appreciated until recently. Formerly, the clay was wet, or fluxed, by the addition of sodium carbonate, borax, or some other mild alkaline reagent to the clay and water mixture. The adhesive was added which was not only expected to develop no plasticity itself but rather was expected to disperse or colloiddally protect the clay in the coating mixture. It has recently been found that the addition of very small amounts of such alkaline wetting agents as tetrasodium pyrophosphate to almost solid masses consisting of 2 parts of clay to 1 part of water (by weight) reduces these mixtures instantly to free flowing suspensions. The function of the added colloid then becomes one of supplying adhesive strength, principally for bonding the clay to the paper fiber and, secondly, for supplying whatever protective colloid action is required to keep the clay evenly dispersed while it is being applied. This latter requirement is small because the agitation, which is present in many sizing processes, is in itself sufficient to prevent the clay from sedimenting.

The more recent practice is, accordingly, to flux the clay with water in a ratio of about 67% clay to 33% water (by weight) by adding approximately 0.2% tetrasodium pyrophosphate (based on the dry weight of clay present). Clays differ in their requirement of pyrophosphate for fluxing. It should also be noted that the inclusion of other pigments in the coating may increase the amount of flux required. For example, free lime in calcium carbonate, a pigment which is frequently mixed with clay, substantially increases the amount of pyrophosphate required to obtain the maximum fluidity, or fluxing action. The adhesive is prepared at a suitable concentration and added to the fluxed clay in a high speed mixer, ball mill, or mixing equipment. Usually, starch is cooked at a concentration of about 20% with water and added to the fluxed clay in the proportion of about 15 to 20 parts of starch (dry basis) to 100 parts of clay. Only a few minutes of agitation are normally required to secure an intimately mixed clay coating, whereas formerly a considerably longer period of ball milling was required when less efficient fluxes were available.

The use of more suitable fluxing agents has not only permitted the use of dextrinous products arising from the treatment of starch with amylolytic enzymes, but has also permitted the use of other modifications of starch with high adhesive strength. Certain types of torrefaction dextrans have been employed (18). Still other modifications have been used in which the starch is reduced in viscosity, probably more by a destruction of the supermolecular forces between the units of the starch than by rupture of primary valences within

TABLE XXX

Characteristics of Starch-Clay Coatings

15 parts of starch to 100 parts of clay; 45% of total solids.

Type of corn starch used as adhesive	MacMichael viscosity	MacMichael yield value	Dennison wax test
Chlorinated	30.5	7.6	3.25
Peroxidized	28.2	1.0	4.50
Special torrefaction, Dextrin A.	28.5	2.5	4.50
Native starch, enzyme-converted	32	3.0	3.75
Residue starch, enzyme-converted	29	0.0	4.50
Acetic acid-treated	31	0.0	3.50

these units. An example of this type of modification is, possibly, the action of relatively high concentrations of strong organic acids, such as acetic acid, on starch. Oxidized starches, other than a chlorinated type have also been used in clay coatings; for example, "peroxidized" corn starch.

The characteristics of clay coatings containing several of the starch modifications mentioned above are given for comparison in the following discussion. The method for preparing the coating and for evaluating its characteristics is essentially as follows: The starch is gelatinized by heating a 20% suspension (by weight) in water to 95° C., with stirring, and maintaining the starch paste at

this temperature for 5 min. The paste is cooled to 37° C. and added to a sample of domestic clay which has been fluxed with 0.2% tetrasodium pyrophosphate in water, a ratio of 2 parts of clay to 1 part of water (by weight) being used. High speed stirring for 10 min. at room temperature is used to obtain intimate mixing. These conditions are satisfactory for the starches listed in Table XXX. When modification of starch by enzyme is employed, the cooking procedure is altered by including in the mixture 0.85% of a commercial liquefying enzyme known as Amyliq, adjusting the pH of the suspension to 7.0, and maintaining the temperature of the starch slurry at 77° C. for 30 min., with stirring, before bringing it up to 95° C. for the 5 min. cooking period.

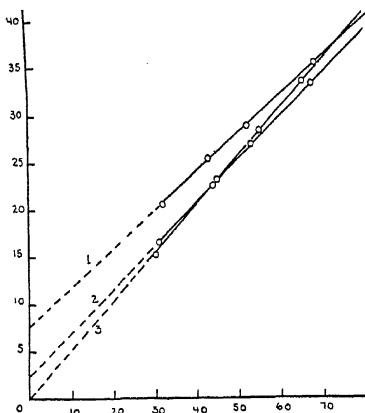


Fig. 96. Viscosity characteristics of starch-clay coating mixtures. Curve 1, chlorinated corn starch; Curve 2, "peroxidized" corn starch; Curve 3, modified, residual starch from a diastase treatment at low temperature. Abscissa, R.P.M. of the MacMichael viscosimeter; ordinate, degrees MacMichael.

The viscosity and plasticity⁶ of the coatings are measured directly after the coatings are mixed, in a MacMichael viscosimeter at 25° C. A No. 26 torsion wire connected to the disk type of plunger is used, and the torque that develops is noted in degrees MacMichael as the viscosity cup containing the coating is allowed to revolve at several different speeds. When the degrees of torque are plotted against R.P.M., a straight line is obtained if the coating has been properly prepared. Exceptions are noted when starch products with inherently high viscosities or abnormal tendencies to gel are used or if extreme limits of speed (R.P.M.) are employed. Since viscosity may be defined as the shear for a given rate of shear, the slope of the curve for a fluid is proportional to its viscosity. A speed of 56 R.P.M. was arbitrarily selected as a unit rate. The MacMichael viscosity then becomes equal to the degrees estimated at this speed. Several typical curves are shown in Fig. 96. It will be noted from the curves that, in

⁶ The writer is indebted to Dr. F. Frost for the methods suggested to evaluate the viscosity and plasticity of clay coatings.

general, clay coatings are not true fluids, since, when their viscosity curves are extrapolated to zero R.P.M., the lines do not pass through the origin. Most coatings exhibit plasticity, which may be estimated from the point of interception of the extrapolated viscosity curve with the axis for degrees MacMichael. This point, when expressed as degrees MacMichael at zero R.P.M., is taken as an index of plasticity of the clay coating and is called the yield value.

A stock of unsized, bleached sulfite paper was procured, and sheets were coated with a common type of laboratory applicator. After the coated sheet was dried and conditioned, the adhesive strength of the coating was evaluated by the Dennison wax test at a fixed temperature and humidity. This test, which involves the application to the coated paper of melted, hot waxes, which are graded by their degree of tack, is frequently used by the paper industry to predict whether the coating will be strong enough, when printed, to withstand the tack of the printing ink impressed on the paper with type or other impressions. The results of the test are expressed on an arbitrary scale, and the higher the number of the wax, the greater is its tack. The values given in Tables XXX and XXXI denote that the coated papers used in the tests showed no removal ("pick") of coating from the paper fiber when a Dennison wax corresponding to the number listed was melted, applied to the paper, and pulled away, standard conditions for the test being used. The fractional values given in some instances indicate the proportion of all duplicate tests that showed no "pick" when the next higher wax in the Dennison series was used. Thus a wax test of 4.5 indicates that in all tests of the coating there was no "pick" on No. 4 wax and in half of the tests no "pick" on No. 5 wax. On this scale of values, a wax test of 4 indicates that satisfactory printing should result from using the coated paper with ink of average degree of tack. A wax test of 3 or lower indicates a weak coating and a wax test of 5 or higher, an excellent coating.⁷

All of the coatings listed in Table XXX were made with 15 parts of starch (dry substance) to 100 parts of clay and a total dry substance of 45% in the coating. These values were adjusted before the final mixing operation by addition of appropriate amounts of starch sol and water. The coatings are all of approximately the same viscosity. A viscosity range of 25 to 35 MacMichael degrees is suitable for brush coating work. The relative plasticity of these coatings, as indicated by the yield values, shows considerable variation. Coatings with yield values above 10 are almost incapable of being spread to make an even coating on the paper by the applicator, whether it is a brush, a metal roll, or an air knife. A yield value between 5 and 10 indicates a passable coating and a yield value below 5 indicates excellent spreading quality. It is to be noted that the coating made with the chlorinated starch shows the highest degree of plasticity when the formulation and method for preparation described above are used. This is surprising, since the starch sol itself, at the concentration used, exhibits

⁷ For additional methods used in evaluating a starch for clay-coating paper, reference is made to the work of Saxl (19). The TAPPI tentative standard for the wax test is given (*Paper Trade J.* (TAPPI), 115, 220 (1942)).

the least plasticity of all the starches tested. This might indicate that the fluxing action of the pyrophosphate and of the chlorinated starch is not an additive action. A similar result is obtained when one attempts to use mixtures of starch and certain proteins as suspension agents for clay. The combined effect is not equal to the sum of their independent effects on clay suspensions. The modified, residual starch ("Residue starch"), which was pretreated with diastase at a low temperature to remove those elements of the starch that contribute most to the plasticity of its paste, makes a clay coating mixture having a minimum of plasticity, as might be anticipated. In addition, this coating exhibits about the maximal adhesive strength of the samples tested. The coating made from starch pretreated with acetic acid also exhibits a minimal plasticity. The action of the acid in the pretreatment is difficult to explain and is probably quite complex. From the results obtained it might be assumed that the crystalline regions of the original granule have been solubilized and that the component molecules have been so modified that their tendency to reorient has been greatly reduced. It is also to be noted that both "peroxidized" corn starch and the special torrefaction dextrin of corn can be used to make acceptable brush coatings by the formulation suggested.

One of the more recent developments in the application of coatings to paper is the so called machine coating process. The essential difference between this method and the one previously described is that, whereas brush coatings are applied to a dried sheet of paper, in the newer method the coating is applied to the web of the paper before it is completely dried. This change eliminates an additional operation and makes coating an integral part of the production line in paper manufacture. However, the application of a coating to an undried sheet requires that a material reduction be made in the water content of the coating suitable for brush work. Coatings containing as high as 60% of total solids are used. The reduction of the water content of the coating to the level indicated requires that a very much smaller amount of water be used for the preparation of the adhesive than is customary in the preparation of the adhesive for brush coatings. Otherwise, insufficient water is available to properly preflux the clay. Hence, for the newer type of coating starch products that are extremely thin or of low viscosity are required. They should be so modified that they will cook to fluid sols with about 2 parts of water. This requirement would seem to prohibit the use of starch, since it is known that when starches are modified beyond the level necessary to produce a satisfactory brush coating there is a sudden and decided decrease in the adhesive strength of the modified starch. This result is particularly true of starches modified by oxidation or by enzymes. Until quite recently it was assumed that casein or an equivalent adhesive was required to prepare a satisfactory machine coating. However, an experiment will be given to illustrate a principle which encourages the view that starch products may find application in this field as well.

The "peroxidized" corn starch mentioned above was used to make three different coatings varying in total dry substance content from 35 to 45%. A

proportion of 15 parts of starch to 100 parts of clay was used in each case. The coatings were prepared and tested by the methods previously outlined and the results are summarized in Table XXXI. This experiment would seem to indicate that as the content of dry solids of the coating is increased the strength of a given adhesive is increased. Stated differently, as water is added to a coating more adhesive is required and conversely as the content of water becomes less, less adhesive is required. Therefore, it seems probable that, if the total solids of the clay coating were increased to 55 to 60%, a satisfactory coating composition

TABLE XXXI
Characteristics of Starch-Clay Coatings Varying in Composition

Type of corn starch used as adhesive	Ratio of starch to 100 parts clay	Per cent total solids	MacMichael viscosity	MacMichael yield value	Dennison wax test
Peroxidized	15	35	10.0	0.0	3.50
"	15	40	15.5	0.3	4.00
"	15	45	30.0	2.5	4.50
Special torrefaction, Dextrin B	12	57.5	39.0	7.0	4.00
" " " "	10	57.5	30.0	3.0	2.00

with only fair intrinsic adhesive properties could be prepared by using a materially reduced ratio of starch product. This probability assumes that the clay will be properly prefluxed with a very efficient agent, such as tetrasodium pyrophosphate. These developments have suggested that a slight alteration in present methods for producing corn torrefaction dextrans, by reducing the paste viscosity under conditions of both a minimal hydrolytic degradation and color formation, would lead to a satisfactory adhesive for this type of clay coating. This type of modification was used for the preparation of the special corn Dextrin A, and the modification was extended to prepare the special corn Dextrin B. Owing to the low viscosity of Dextrin B, its use made a clay coating with a low adhesive strength at a total solids content of 45%, even though a ratio of 15 parts of dextrin to 100 parts of clay was used. However, as the results in Table XXXI show, a satisfactory coating was made with 12 parts of dextrin to 100 parts of clay in a coating mixture containing 57.5% of total solids. The coating was found to be weak when 10 parts of dextrin to 100 parts of clay were used in a coating mixture containing 55.7% of total solids.

From the discussion it might be presumed that the adhesive strength of the coating could be increased indefinitely by increasing the ratio of starch product to that of clay, other factors remaining constant. By this procedure one should be able to obtain coated papers which would give very high indices for printing by the Dennison wax test. The difficulty encountered in this procedure with starch products is that, if the concentration of a starch product is increased above a critical limit in respect to the amount of total water present, the coated paper

does not develop the desired gloss when it is ironed (calendered) by the finishing rolls.

In recent years, considerable effort has been devoted to improving the manner of applying clay coatings, particularly the machine coating operation. Although most of these improvements in mechanical details are not fully disclosed, it would appear that the general trend has been to design coating applicators which will satisfactorily spread coatings exhibiting a substantially higher viscosity and more plasticity than the limits imposed by older processes. For example, it is possible to apply coatings in the newer machine applications, with coating viscosities of the order of 10,000 to 50,000 centipoises, whereas the viscosities of coatings described in the preceding sections are of the order of 500 to 5000 centipoises. These improvements in machine design have also favored the possible utilization of starch products in machine coatings. The special torrefaction Dextrin B mentioned above, when used at as high a ratio as 20 parts of dextrin to 100 parts of clay, produces a coating containing 67% of total solids which has a viscosity of the order of 50,000 centipoises. When this coating is applied to paper, it shows an adhesive value corresponding to a Dennison wax test of 8 to 9. As a result of these developments, some mills also find it possible to use chlorinated corn starch mixed with some casein, while others are attempting to use the chlorinated starch alone as the adhesive in machine coatings.

In the newer type of application of the coating, some thixotropy in the coating is permissible, even desirable. When these relatively thick-bodied coatings are metered out to the paper sheet, it is an advantage for the coating to become more fluid under the pressure exerted by the applicator. A dilatant coating, on the other hand, is obviously not desirable. A thixotropic coating is recognized by a curvature downward in its viscosity curve as obtained from measurements made by methods similar to those previously described. The curvature in the viscosity graph may be taken as a measure of thixotropy. This value is indicative of the rate of change in the viscosity for a change in the rate of shear, since the tangent to the curve is proportional to the viscosity. For convenience, the viscosity at some specified low rate of shear divided by the viscosity at a specified high rate of shear may be taken as a "thixotropic index."

The use of starch modified by enzymes as an adhesive in machine coatings seems to involve considerable difficulty, unless a radically new technique is developed for such modification. In practice, starch is liquefied by amylases by gelatinizing the raw starch in the presence of the enzyme. Even partial gelatinization of untreated corn starch with 2 to 3 parts of water results in a rigid mass. Owing to its poor thermal conductivity, it cannot be heated further without an undue amount of local overheating. If the enzyme were present, it is certain that large portions of the enzyme would be continuously inactivated by this overheating until its activity was completely destroyed. It would be necessary, therefore, to perform practically all of the liquefaction below the gelatinization temperature of the starch. Although such a conversion is possible, at least at a low rate, the reaction is predominantly saccharogenic, as has been

pointed out by Kerr and coworkers (15). The author has attempted to extend conversions of the type indicated to the degree at which the product could be used in machine coating formulations but has found in each case, as might be anticipated, that the coating prepared is definitely low in adhesive strength.

Sheets (20) has recently presented a critical study of the structural viscosity of coating clay compositions. The results reported should aid an attempt to evaluate the effects of various starches for this use particularly in affording an understanding of variables introduced into the tests by the type of clay and the type and amount of dispersing or fluxing agent used. Several coating clays were studied, and it was established that the differences in the flow properties of the clays are caused by differences in the distribution of particle size in clay species, and in hydrating ability. However, the amount of dispersing agent, such as $\text{Na}_4\text{P}_2\text{O}_7$, used is the most important single variable in altering the flow properties of the clay suspensions studied. When enough dispersing agent has been added to give a minimal viscosity to the clay slip, the next most important variable is the particle size of the clay. Washing the clays with acid tends to make them more non-hydrous. A mild heat treatment irreversibly dehydrated one sample sufficiently to change its flow properties to some extent. Nevertheless, neither acid washing nor heating, singly or in combination, was found to be sufficient to change the flow properties of one clay to those of another.

It was established by Sheets that an intense thixotropic build-up occurs in clay suspensions when certain amounts of dispersing agent are used. The amount of dispersing agent required to give this thixotropy is slightly less than the amount required to give a minimal viscosity to the clay slip. Clays with relatively large particle size may show a marked dilatant behavior over a relatively narrow range of clay concentration when enough dispersing agent has been added to these clay slips to secure a minimal viscosity.

For fractions of clay from the same species, the general conclusion may be drawn that clays of smaller particle size give a thinner suspension for a given solids content than clays having a large particle size. This is particularly true for concentrated clay suspensions such as are used in machine coatings. This result should be considered, when it is desired to use the less expensive modifications of starch as the adhesive in machine coatings.

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near future. It is anticipated that in 1944 the fermentation industries will be using grains at the rate of 6 million tons or more per year, but current developments now in the laboratory stage might conceivably increase this consumption by 50%. Thus, starch has become the principal raw material for fermentative processes, and blackstrap molasses has accordingly become relatively less important.

2. Relation between Agriculture and the Fermentation Industries. In order to understand the significance of the development mentioned above, and before a conclusion as to the permanence of this change can be reached, it is necessary to examine the interrelationship of several agricultural activities. This examination will not present all of the information necessary to form this conclusion, but it is the first and most important step in the evaluation of the basis on which this new chemical program rests.

Throughout the scientific and other literature there are found many excellent analyses of the agricultural situation which have a bearing upon this subject. The studies by Hale (2), Christensen, Hixon, and Fulmer (3), Shepherd, McPherson, Brown, and Hixon (4), Willkie and Kolachov (5), and Filley, Loeffel, and Christensen (6) have sketched the broad outlines of agriculture and animal husbandry upon which the recent developments in the fermentation industries must stand if they are to become a permanent part of American economy. Essentially, the situation is that, in order to produce meat, dairy products, and poultry products in an efficient and economical manner, it is necessary to feed rations containing, on the average, about 1 part of protein for 6 parts of carbohydrate. However, feed grains, such as corn, barley, rye, sorghums, and oats, contain 1 part of protein to 10 parts of carbohydrate; that is, they contain either too little protein or too much carbohydrate. To correct this unfavorable ratio protein may be added or starch may be removed. In the past, in addition to domestic supplies, about a million tons of protein concentrates have been imported each year in an effort to correct this protein deficiency, and animal husbandry men report that this amount has not been sufficient. Since such feedstuffs cannot be imported uninterruptedly, an alternative procedure, the removal of some of the excess starches, has recently been used to a larger extent than formerly.

It is interesting to attempt to compute the magnitude of this indicated starch surplus. To produce a proper balance of protein and carbohydrate in the animal feeding ration at least 10 lbs. of a 30%, or higher, protein concentrate should be fed with 90 lbs. of feed grains. Each year nearly 100 million tons of feed grains are consumed in the United States. Thus, to obtain the desired balance 11 million tons of protein concentrates should be added. It is estimated that about 6 million tons can be supplied by alfalfa, oil cake meal, packing house wastes, and other present sources, which would leave 5 million tons to come from new sources. If we assume that this protein concentrate (30% protein) is derived from grains which contain 10% protein and 60% starch, it is evident that a quantity of 10 million tons is the amount of the indicated normal starch surplus at present.

This surplus has been used in the past to some extent by the corn-milling and the fermentation industries. However, by far the greater proportion of the estimated surplus has been wastefully consumed in improper feeding, the starch being converted into an excessive amount of fats or eliminated in the excreta. Therefore not all of this starch can now be said to be available, but it is to be noted that farmers are rapidly adopting better feeding practices for their livestock and that the starch surplus is steadily becoming more evident. The assumption is made that education in respect to animal feeding will continue and that the indicated starch surplus will continue to materialize and supply the technical and economical basis for the development of fermentation and other industries that use starch. It is essential, however, that these industries be carefully coordinated with the agriculture which supplies the starch and uses the residual proteins. Among obvious reasons why this coordination is essential is that it may provide for the maintenance of soil fertility and of sound land use practices.

3. Ethanol. It is quite probable that one of the first organic chemical industries was the production of ethyl alcohol. Because of the length of time that the industry has been in existence, the impression may prevail that this industry is at or near the peak of manufacturing perfection. Beresford and Christensen (7), Willkie and Kolachov (5), and Christensen (8) have shown that this conclusion is in error. The manufacture of ethanol from starchy substrates by procedures commonly used at present is inefficient and can be considerably improved in many respects.

One obvious improvement is the elimination of barley malt by some less costly and more effective source of amylase used to convert starches to fermentable sugars. At present, 8 to 10 lbs. of barley malt, costing three times as much per pound as does grain, are used with each 92 to 90 lbs. of grain. Assuming a yield of 5 gals. of ethanol per 100 lbs. of total grain, the amylase of barley malt costs 4 cents per gallon of ethanol produced, which is one-fifth of the normal selling price of the alcohol. Underkofler, Fulmer, and Schoene (9), Hao, Fulmer, and Underkofler (10), and Beresford and Christensen (7) have described a fungal amylase that can be made in the plant and which costs less than 1 cent per gallon of ethanol.

Willkie and Kolachov (5) have pointed out the waste of steam, power, and water which is involved in the present plant operating practices, and throughout the fermentation industries engineers are making rapid progress in reducing the costs of factory operation. These savings may eventually amount to several cents per gallon of ethanol.

Beresford and Christensen (7) and Christensen (8) have pointed out another serious inefficiency. On the assumption that 1 mole represented by $C_6H_{10}O_5$ should theoretically yield 2 moles of ethanol and 2 of carbon dioxide, each pound of alcohol (C_2H_5OH) produced represents 1.761 lbs. of starch consumed. All of the balance of the raw material processed should be recoverable as residual solids; that is, the weight of the residual solids, plus 1.761 times the weight of ethanol, should equal the weight of the dry matter which enters the process.

An examination of published data shows that only 75 to 90% of the dry matter can thus be accounted for, and the indications are that the loss is in carbohydrate and that 15 to 30% of the starch is lost; possibly it is converted to carbon dioxide and water. The economic loss thus incurred may reach a sum of 6 cents per gallon of ethanol.

Research on the manufacture of ethanol is now very active and it can be expected that presently its scientific and economic status will be markedly improved. These improvements also should be considered, as well as the potential surplus of starch discussed previously, in reviewing the economic basis upon which a large permanent starch-alcohol industry may be built. Major changes from orthodox practice and in plant design are in progress or are contemplated. Several plants now avoid grinding the grain and use a short steeping period preceding the cooking operation, which may be discontinuous or continuous. Continuous cooking is preferable, because it reduces the steam consumption and it increases the quantity of material handled per unit. It is probable, however, that a coarse grinding of the grain will be used, because it is an inexpensive operation and it allows a precooking treatment which results in a definite improvement in yield.

A partial liquefaction of the starch prior to cooking is becoming a general practice. Bacterial amylases are preferred for corn and sorghum starches, since they have the desired thermostability, but barley malt may be used satisfactorily with wheat, because of the lower temperature of the range of gelatinization of wheat starch. Careful temperature control is essential to success in this operation. The precooking treatment may be applied to fine or coarsely ground grains, if sufficient time for heat and moisture penetration into the coarse particles is allowed. The treated grain slurry should be cooked in a continuous process, and the present trend is toward a relatively high cooking temperature, above 150° C., and a relatively short cooking time of from 1 to 5 min. As a means of reducing the steam consumption, a high mash concentration should be used, approaching and possibly exceeding $\frac{1}{2}$ part of ground grain to 1 part of water.

Flash-cooling, which permits the use of the steam again, and dilution with cold water constitute a preferred practice to reduce the temperature of the mash prior to adding the second lot of amylase. The enzyme used may be a mixture which includes a small addition of bacterial amylase at above 80° C. and a cereal or fungal amylase at 55–60° C. (The functions of α - and β -amylases in this process are discussed in previous chapters by Kneen and by Severson.) The mash should be cooled immediately to the temperature of fermentation, especially if a fungal amylase preparation is used. Also, the concentration of the mash going to the fermenter should be $\frac{1}{4}$ part of grain to 1 part of water, or possibly a higher ratio. The saccharification technique outlined, particularly the addition of the bacterial amylase above 80° C. and the rapid cooling of the mash, prevents the irreversible change that takes place in the starch when cooked mash is slowly cooled to below 80° C. according to the procedures commonly used at present. Larger yields of both ethanol and residual solids can thus be obtained,

and a recovery of products closely approaching 100% of the theoretical value results.

Fungal amylase and bacterial amylase can be made at larger alcohol plants to secure the savings indicated, and possibly the relatively small plants may find it economical to produce their requirements of this enzyme. The bacterial amylase may be produced in a thin stillage, under carefully controlled conditions. At present, it appears to be preferable to produce the fungal amylase by growing a selected mold, *e.g.* *Aspergillus oryzae*, on the brans which are removed from the grain in milling, but liquid substrates may be employed.

By the use of the processes proposed, ethanol yields from corn and sorghum grains are about 38 g. per 100 g. of total dry substance in the grain bill, or 11.50 proof gals. per 100 lbs. of dry substance used. This yield is about 15% above the value commonly reported for the older processes; a recovery of 33 g. of dry residual solids per 100 g. of dry substance processed may be compared with 26 to 30 g. obtained previously. As a result of the changes in process outlined, the steam consumption, including that for drying the residual solids, should not exceed 50 lbs. per gallon of alcohol, and all other factory operating costs may be reduced. Solid and liquid CO₂, vitamin concentrates, supplemental protein feeds, oils, and glycerol are the principal by-products from the ethanol fermentation, and provision should be made that they are fully recovered. Rigid chemical control should, of course, be exercised in all phases of the plant operation.

4. Butanol and Acetone. Early during the first World War, the British Admiralty was faced with a serious shortage of acetone for cordite manufacture. The small production of acetone in scattered wood distillation plants could not meet the demand. Additional sources of acetone were sought, and it was found that a commercial production of *n*-butanol and acetone, in relatively small quantities, was being carried out at Rainham, England, by the fermentation of potatoes by a process developed by Fernbach, of the Pasteur Institute. The *n*-butanol and acetone were being used as the raw materials for the manufacture of synthetic rubber by the Matthews sodium polymerization process. The history of this industrial program has been described by Perkin (11), Gabriel (12), and others.

The supply of cull potatoes in England was small and the operation was transferred to Canada. A Toronto grain distillery was remodeled to make *n*-butanol and acetone from corn. Later, another factory was established at Terre Haute, Indiana, by British and United States governmental agencies. This plant was subsequently transferred to private ownership and it has since become one of the principal sources of these fermentation chemicals.

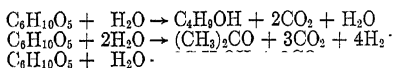
Until about 1935, attempts to use blackstrap molasses as the raw material instead of grain for this fermentation were unsuccessful, but research finally developed modifications of the basic process that permit the substitution of blackstrap molasses for grain, and for several years molasses was the principal raw material for this fermentation industry. In 1942, molasses became unavailable as mentioned above, and this industry again turned to the use of grains.

A considerable amount of research has been carried out on a study of the fermentation of xylose solutions from agricultural wastes, but no successful commercial application has resulted. The report of Bryner, Christensen, and Fulmer (13) describes some of the problems in this procedure.

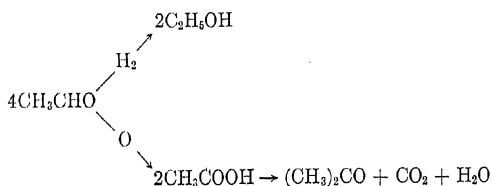
Both *n*-butanol and acetone have been made synthetically, but the fermentation industry has been able to meet this competition and as additional by-products are developed the economic status of the fermentation process is improved. At present, methanol is made from the hydrogen and carbon dioxide produced in the fermentation, and vitamin concentrates are made from the stillage.

One of the principal faults of the butanol-acetone fermentation is the low mash concentration that must be employed. A final concentration of "total solvents" (60 parts of *n*-butanol, 30 parts of acetone, and 10 parts of ethanol by weight) of 2.2 to 2.5 g. per 100 cc. is about the maximum that can practically be obtained. The bacteria used, strains of the species generally known as *Clostridium acetobutylicum*, produce an amylase that permits the use of starchy substrates directly, without the saccharification step which is needed in the ethanol fermentation. A high steam consumption is the inevitable result of the low mash concentration, and although continuous cooking of the mash can improve this situation, distillation and recovery of the residual solids require the use of a large amount of steam.

The yield of "total solvents" from corn varies in practice from 26 to 28 g. from 100 g. of dry substance treated, or about 36 to 39 g. from 100 g. of starch. Normally, the "total solvents" consist of 60 to 62% of *n*-butanol, 29 to 31% of acetone, and 8 to 11% of ethanol by weight. Little is known concerning the mechanism of the fermentation, although extensive research has been done by several groups of workers to discover the chemical processes involved. However, the process may be illustrated by the following general reactions:



Acetic and butyric acids have long been known to be intermediates which accumulate during the first part of the fermentation and disappear during the last stages. Many investigators have found evidence that acetaldehyde is an important intermediate and traces of formic acid have been isolated. Severson and Christensen (14) found that *n*-butyraldehyde is quantitatively reduced to *n*-butanol when it was transfused into an active corn mash fermentation. Acetaldehyde yielded acetone and ethanol, as follows:



Severson (15) reported that acetic acid is converted to acetone, while butyric acid yields a mixture of *n*-butanol, acetone, and ethanol in a ratio not unlike that obtained from starch. Formic acid is converted with difficulty to CO₂ and H₂.

Christensen (16) found that an increase in the hydrogen pressure and a simultaneous removal of CO₂ increase the proportion of *n*-butanol formed. Johnson, Peterson, and Fred (17) found that the use of a reduced sugar, mannitol, results in the production of a larger proportion of butanol than is obtained from starch.

A most interesting result is found in the reports on the subject of "sluggishness." Frequently it has been observed in practice that, although fermentations start normally, the acidity fails to decrease ("break") and the fermentations end abruptly with the formation of a large amount of residual carbohydrate, a high concentration of acetic and butyric acids, and a low yield of "solvents." Legg (18) described the causative factor as a filtrable, reproducing, thermolabile one, resembling a bacteriophage in some respects, and described a technique of acclimatizing or of selecting a culture to obtain an immune strain. Starr (19) further described the effect and confirmed the work of Legg. Starr also showed that the filtrate did not produce lysis or otherwise induce changes in morphology, but that it did affect the metabolic processes of the bacteria.

5. Glycerol. The production of glycerol by yeasts as a by-product in alcohol fermentation was first noted by Pasteur, and although means for improving the yield have been developed, there has not been a large commercial production of glycerol by this process because of the high cost of recovery from the fermentation mixture. However, during the first World War, Germany used this process for the production of glycerol. There is at present considerable interest in the production of glycerol by new methods, particularly in the United States, since the yield from soap manufacture has been unable to keep pace with the increasing demand for the product.

Connstein and Lüdecke's investigations (1915) (20) on alcoholic fermentation indicated methods to increase the yield of glycerol to values which were of commercial interest. Pasteur and others had reported no more than about 3 g. of glycerol from 100 g. of sugar in the normal fermentation procedure. Neuberg and Reinfurth (1920) described three general courses for the fermentation of sugars by yeast:

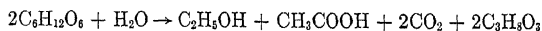
1. Normal, in the presence of air and in acid media,



2. In the absence of air and in the presence of sulfites,



3. In alkaline media,



Connstein and Lüdecke (20), Cocking and Lilly (21), Ling (22), and others have reported additional details of the several variations of these basic processes. The alkaline process gives yields of 10 to 15 g. of glycerol per 100 g. of sugar, and large amounts of alkali are required. The sulfite process yields 35 g. of glycerol per 100 g. of sugar and requires a weight of sodium sulfite as much as (or more than) the weight of sugar which is initially present in the medium. In most of the proposed methods for the recovery of the products, the sodium sulfite is not reclaimed, but instead is removed by precipitation of the sulfite (with CaCl_2) as the calcium salt and by a steam distillation of the glycerol from the solution which contains the resulting sodium chloride. For a critical discussion of the details reference is made to the report by Nord (22a).

The cost of using the indicated large amounts of alkali or of sodium sulfite, including the high recovery cost which results from their presence, has led to research for the development of a fermentation process better suited to commercial use. Recently, progress has been made in this connection but, because of the secrecy demanded by the military importance of the results, it may not be reported at this time.

Except for the recovery operation, the manufacture of glycerol from starch substrates requires practically the same equipment as that employed in the production of ethanol from the same raw material.

6. Butylene Glycol. Because a large portion of the information developed by current research on the production of 2,3-butylene glycol is of military importance, it is not possible to present here an adequate review of the more recent work. The principal interest in the subject is, of course, the dehydration of the product to form butadiene for the production of synthetic rubber. However, the product is of interest because of many other uses for the glycol as well.

Butylene glycol, or its partial oxidation products, acetylmethylcarbinol and diacetyl, have long been known as minor products of several bacterial fermentations of starch and sugars. Kluyver and Scheffer (23) described means for improving the yield of the glycol and for its commercial production. Breden and Fulmer (24) studied the production of the glycol and the carbinol by a number of species of *Aerobacter*. Fulmer, Christensen, and Kendall (25) reported the influence of the sugar concentration and other factors upon the yield of the glycol from sucrose and reported yields as high as 50 g. per 100 g. of sucrose, or nearly the theoretical yield of 1 mole of glycol from 1 mole of the hexose sugar.

Two bacteria are of particular interest for this fermentation. *Clostridium polymyza* produces an amylase and thus can ferment a starch substrate directly to produce a mixture of the glycol and ethanol in a ratio of approximately 1.5 to 2.0 parts of glycol to 1 of the alcohol, by weight. *Aerobacter aerogenes* produces only a little amylase, and the mash should therefore be saccharified as for fermentation by yeast. It produces the *meso*-glycol, variable amounts of lactic acid, and sometimes a small amount of ethanol. By a proper control of the sugar concentration, pH, and mineral nutrients, lactic acid and ethanol production can be held at very low values. Some cultures of *Aerobacter aerogenes* ferment starch

substrates directly, since no added amylase is required, but even in such cases it is desirable to add some other amylase or to cook with dilute mineral acids if for no other reason than to reduce the viscosity of the mash.

The glycol may be recovered by steam distillation, by somewhat the same procedure as is used for glycerol recovery, or it may be extracted with *n*-butanol, ethyl ether, or some other suitable solvent. If extraction is used (and this is the more economical process), it is desirable to mill the grain before fermentation and to start with a mash containing only a small amount of non-starchy materials. Filtration after the fermentation is difficult and expensive because of the dilution caused by the washing of the insoluble material. The fermented mash should be concentrated to 20 to 22 g. of glycol per 100 cc. before extraction.

7. Lactic Acid. Until quite recently, most of the lactic acid used in the United States was imported. Dark lactic acid has long been used for deliming hides in leather manufacture and the light edible grades have had a limited use in the production of food products. There is currently a great interest in lactic acid, or aliphatic lactates, as a source of acrylate plastics, and this has given a decided impetus to research on its commercial development.

At present it is customary to use as a substrate a starch-rich fraction from corn milling, and to saccharify it by cooking the fraction with a dilute mineral acid, sometimes following this procedure with the addition of an amylase. Several of the species of *Lactobacillus* are used for the fermentation, and neutralization of the acid with sodium carbonate or lime is used to maintain the desired pH. Edible lactic acid is readily recovered by a liquid extraction of the fermented mash which has been acidified with sulfuric acid, and the extract is concentrated and then steam-distilled. The dark impure grades are made by neutralization of the acid with lime, liberation of the lactic acid from its calcium salt by the addition of sulfuric acid, and concentration of the acid to the desired value. This may be followed by a clarification and decolorization procedure.

A mixed culture of thermophilic bacteria is able to ferment starchy substrates directly, and therefore a low cost for conversion by this means is indicated, but, as far as is known, no commercial application of this process has been made.

8. Other Fermentation Chemicals and Processes. Citric and gluconic acids are made by the action of fungi upon sugar solutions. Edible grades of acetic acid are produced from dilute ethyl alcohol solutions by bacterial oxidation. Fermentation processes are important in the manufacture of many food products, the preparation of tobacco, and in the retting of fibrous plant stalks for separation of cordage and fibers. Current research has shown many possible applications of the delicately balanced oxidative powers of the species of *Acetobacter*. A discussion of these interesting and important or potentially important processes is not properly a matter for consideration here.

9. Present and Future Markets for Fermentation Chemicals. A detailed analysis of the markets and possibilities for future markets for the fermentation chemicals cannot be presented here. However, several of the more important probable markets will be noted. The requirements for ethanol in the several

peace time markets have expanded greatly as a result of present military needs, and in particular large volumes are required for the manufacture of smokeless powder. The use for plastics has expanded considerably and further expansion is likely, especially for the manufacture of cellulose acetate from acetic acid and its anhydride, of ethyl cellulose, and of the polystyrenes.

The greatest of the new demands is for synthetic rubber. Fulmer (26) has reviewed the history of synthetic rubber and has shown that the extensive synthetic rubber industry existing has largely been developed upon the basis of its production from butadiene made from alcohol. Practically all of the Russian production, which unofficially is reported to have reached 90,000 tons in 1939, has been made from fermentation alcohol produced from grains and potatoes. Three general methods of preparation have been employed. In one, the ethanol is passed in a vapor phase over a combination dehydrative-dehydrogenative-coupling catalyst, to yield butadiene, butylenes, ethyl ether, and many other products (27). In another, ethanol and acetaldehyde are passed over a similar catalyst in a vapor phase, in a so called two-stage process (28). The third, called a three-stage process, involves preparation of acetaldehyde in one step and of ethylene in another and finally coupling the two in the third. Because of the secrecy demanded for military reasons, little is reported concerning the current developments in this industry. It is known, however, that a substantial proportion of the American requirements for synthetic rubber will be filled by the use of butadiene made from fermentation alcohol (29). The extent of these requirements is based on the estimated amount of production by the synthetic rubber plants now completed and under construction in the United States and Canada. It is interesting to note that to fulfil these requirements would necessitate the production of about 300 million gals. of butadiene and that it would require probably 3 times the total amount of ethanol produced yearly in the United States in normal times to make this amount of butadiene (29). Obviously, the source of a large share of American synthetic rubber is petroleum products. It may be noted, furthermore, that the best commercial procedures for the production of butadiene from alcohol result in a yield of 70% of the theoretically calculated value. Several research workers suggest modifications which indicate that it may be possible to increase this yield to 85%. Whether the requirement for synthetic rubber indicated or a part of this amount will become a permanent one is a matter for speculation, since the result will depend upon military, technical, and commercial developments which are in progress.

German production of synthetic rubber is based on three basic sources of raw material, farm crops, oils, and coal, which converge at the intermediate, acetaldehyde. The acetaldehyde is converted to 1,3-butylene glycol in one of several different ways and this is dehydrated to butadiene by vapor phase catalysis. The yields by these processes are high, but it would seem that the production costs might also be large. Presumably, all three sources of raw material are used; the proportion of each employed changes according to the supplies available.

Ethanol is made in Germany principally from cull potatoes, sugar beets, grains, and wood sugar.

The Polish, Czechoslovakian, and Italian synthetic rubber industries use ethanol and according to available information employ methods similar to the Russian. The Japanese have also produced synthetic rubber, and many reports give ethanol as the source of the butadiene.

The use of alcohol as a fuel for internal combustion engines has been the subject of extensive research. Christensen, Hixon, and Fulmer (3), Jacobs and Newton (30), Shepherd, McPherson, Brown, and Hixon (4), and Beresford and Christensen (7) have adequately reviewed the technical literature in this field.

2,3-Butylene glycol is of interest in many synthetic reactions in organic chemistry. The *levo* form has properties which recommend its use as a non-evaporative antifreeze. The immediate interest, however, is in its use as a source of butadiene. It may be dehydrated directly by a vapor phase catalytic process, but at present better yields are obtained by the pyrolysis of the double acetate. Elder (31) reported that in this manner an over-all conversion of 88% has been obtained in practice, and the indication from this report is that this yield may be increased.

In conclusion, it seems probable that the principal markets for the fermentation chemicals are for the manufacture of synthetic rubber, in which ethanol and 2,3-butylene glycol are used; for plastics, in which ethanol and lactic acid are used; and for motor fuel, in which ethanol is used. These markets are all potentially large, and the production of the chemicals which may be used to manufacture the products mentioned should provide a profitable manner for the utilization of starch and starch products.

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CHAPTER XX

USE OF STARCH AND STARCH PRODUCTS IN FOODS

A. G. OLSEN, T. J. OTTERBACHER, AND RALPH W. KERR

1. Introduction and General Discussion. The food industries comprise one of the largest consumers of starch and starch products.¹ In addition, about one-sixth of all starch sold for food purposes is purchased by the housewife in the form of small packaged products for domestic cooking. Not only are purified and treated starches used for such varied purposes as starch puddings, confections, salad dressings, pies, and other bakery products but products derived from starch such as sirups and refined sugar are used to a large extent in a host of additional foods as well, such as ices, ice cream, cake, and bread. The manufacture of baking powder consumes a large portion of the starch sold for food purposes. Furthermore, as is generally known, a very considerable tonnage of dry milled flour is used for baking bread, cake, muffins, and similar foods. These flours include those of wheat, rye, corn, and buckwheat. Wheat flour is used extensively in the United States.

Starches and derived sugars provide a large proportion of our calorific requirements. They are energy foods, and the dietary function is the same whether the pure starch is added as such to thicken a soup or a pie filling or to produce a delectable pudding, or eaten in its natural association with other food elements such as the flour used to make bread. 1 lb. of starch provides 1800

¹ The pounds of corn starch and corn starch products used annually for foods exceed that for all other uses combined.

calories, which is about 50% of the daily energy requirements of a hard working laborer. It is evident that the 300 million lbs. of pure starch utilized each year for food purposes provide a more than negligible portion of the nation's caloric requirements.

One of the principal reasons for including pure starch in food products is the effect it has on the texture or consistency of the prepared food. Accordingly, its contribution to the dietary requirements may be overlooked by the consumer.

The starches used for food prior to 1943 were corn, tapioca, potato, wheat, rice, sago, and arrowroot. The total quantity used for food in the United States increased by nearly one-half from 1933 to 1936 and then declined somewhat in 1937. The proportions of the various starches used changed only slightly. The relations between the starches, expressed in percentages of the total quantities used for food, are shown in Table XXXII.

TABLE XXXII

Starches Used for Food: Relative Consumption, by Kinds, 1933 and 1935-37

Kind of starch	1933	1935	1936	1937
Total quantity used for food, <i>millions of pounds</i>	228	254	339	292
Corn starch, <i>per cent of total</i>	86	86	87	82
Tapioca, <i>per cent of total</i>	11	11	10	14
Other starches, <i>per cent of total</i>	3	3	3	4

Source: Compiled from questionnaires returned to the Tariff Commission by distributors of starch.

Of the corn starch used for food, about 80% is sold by the wet milling industry in bulk, and the remainder in packages for retail sale by grocers. Some of the starch sold in bulk by the manufacturers is repacked before the final sale for household use, either as plain starch or as the major ingredient in prepared pudding mixtures. The distribution of sales of corn starch for food is shown in Table XXXIII. Data for the distribution of tapioca for food similar to those for corn starch are not available, but the major portion is sold in package form.

Consumption of corn starch by the brewing industry became very large in the United States after the repeal of the prohibition act in 1933 and accounted for almost all of the increase in the total sales of corn starch for food in this country. Sales to brewers, which amounted to less than 2 million lbs. in 1929, rose to 98 million lbs. in 1938. Corn starch is practically the only kind of starch used by brewers in the United States. It is prepared especially for them in the form of small particles called "grits." Other substances that are used for the same purpose are brewers' rice, a large part of which has been imported, and corn meal, grits, and flakes, manufactured by the dry milling industry.

Most of the starch used in making baking powder is corn starch. Annual requirements have been fairly stable for the last 10 yrs.

The corn starch used for food purposes in the United States of America amounts to well over 80% of the total starches used for such purposes, and in 1939 amounted to 294,956,000 lbs. It is estimated that the amount sold directly through grocery stores for household use is approximately 50 million lbs., and close to 10 million lbs. were sold in the form of prepared dessert mixtures.

The use of corn starch by the domestic or professional cook ranges from the simple thickening of gravy to the preparation of pies and puddings. For most of these purposes other starches such as wheat, rice, or potato can be substituted; in fact, in some of the European countries potato starch is largely used for such purposes. Some exceptions to this direct substitution are discussed under

TABLE XXXIII

Corn Starch Used for Food: Distribution of Sales, by Industries, in Specified Years, 1929-39

The values are stated in thousand pounds.

Item	1929	1933	1935	1937	1939
Sold in bulk to:					
Brewers (refined grits).....	1,282	17,722	62,978	75,435	112,857
Baking powder manufacturers.....	49,719	62,613	58,522	57,039	63,088
Confectioners and confectioners' supply houses..	32,799	29,701	26,335	28,283	29,825
Bakers, bakers' supply houses, flour millers, and mixers.....	30,827	22,206	22,491	24,244	26,573
Dealers and repackers*.....	15,994	12,342	7,058	9,229	9,727
Sold in packages†.....	43,922	50,842	41,329	44,195	52,886
Total used for food.....	174,543	195,426	218,713	238,425	294,956

Source: Compiled from reports of the Corn Refiners Statistical Bureau.

* 20% of the starch reported as sold to dealers, repackers, and dextrin manufacturers.

† 30% of the starch reported as sold to grocers (packages).

tapioca starch, but it is generally assumed that the type of starch to use is determined by availability and price rather than by the character of the starch. It should also be recognized that for many culinary uses packaged starch comes into direct competition with wheat flour. The latter, which after all is close to 80% starch, is actually preferred by some cooks to thicken gravies, pie fillings, etc. In most cases this preference for wheat flour may be due to the natural extension of utility of this ever present staff of life. Some recognition should be given, however, to the fact that starches do differ in flavor, as is readily demonstrated by a direct comparison of the flavor of cooked wheat, potato, corn, and tapioca starches.

The type of pudding produced through the use of corn starch has a rather velvety and "short" texture, as distinguished from the gelatinous or "long" texture produced by tapioca starch. Corn starch is, therefore, preferred for puddings of the *blanc mange* type and is customarily used in packaged, prepared pudding mixtures, most of which, regardless of flavor or color added, would

belong in that general classification. A typical formula for a prepared pudding mixture contains 25% of corn starch, 65 to 75% of sugars, 1% of salt, plus color and flavor as desired. In preparation, the housewife combines the contents of a package with a measured portion of milk. The product is made up so as to give about 4.0% starch in the finished pudding.

In the period 1933 to 1937 the importation of tapioca grew from 190,402,000 lbs. to 382,858,000 lbs., and in that same period the use of tapioca for food purposes grew from 25,702,000 lbs. to 41,457,000 lbs. of which about 13 million lbs. were sold for household purposes through the grocery stores. Nearly all of this came from the Dutch East Indies where, prior to World War II, a high quality *Cassava* starch was produced. A high quality of tapioca has been imported from the Dominican Republic, but this has not been available in any large quantity. Brazilian production in the past, while substantial, has been of too variable quality to be of interest to food manufacturers.

"Tapioca flour" is the misleading, but accepted, commercial designation for *Cassava* starch. The precooked products sold for culinary purposes are referred to as flake or pearl tapioca, depending upon whether the product is the granular quick-cooking minute type or the large round pearl type which requires soaking for several hours prior to cooking.

The granular so called "flake" is produced both in this country and in Java. It is made by heating the moist flour (starch) on iron plates while it is stirred. After it is heated to the desired temperature, the stirring causes the gelatinous starch to "flake" away from the hot plate in the form of tough sheets. This gelatinous flake is dried, granulated to the desired mesh size, and is then ready for use in the household. Continuous processes for this type of product have been developed both in this country and in Java.

The large round pearl tapioca is imported from Java. To make tapioca pearls and seeds, the starch, after being broken up in the drying shed, is sieved and transferred to hammock-like contrivances suspended from the roof; a rocking motion imparted by hand to these hammocks causes the starch grains to adhere to one another. The particles are then graded as to size by screening, cooked in the same way as for flakes and siftings, again screened, and finally dried. This description applies particularly to manufacture in the small factories of Java where natives make most of the tapioca products exported. In the larger factories, owned principally by Europeans, the equipment is of more modern design and appropriate for production on a larger scale than in the factories operated by natives of Java. Such a process, of course, requires much cheap native labor. No successful commercial equipment for producing this product has been put in operation in this country.

The quality of the imported tapioca flour varies considerably. Some of these variations may be due to the character of the starch itself, but in most cases low quality means excess fiber or high ash, both of which indicate poor starch separation. Important considerations from the food manufacturer's standpoint are color, viscosity, body, temperature range of swelling, freedom

from dirt, and fiber. Typical analysis of different *Cassava* products are given in Table XXXIV. The meal is not usually available in this country, but is used extensively for food purposes by the native population wherever *Cassava* is grown.

For comparison with other starches, potato, wheat, corn, etc., we have from Winton these ranges: protein 0.1 to 1.8%, fat 0.02 to 0.2%, fiber 0.02 to 0.19%, ash 0.02 to 0.7%.

Many attempts have been made to prepare flaked products, similar to minute tapioca, from the plain corn, potato, and other normal starches. These attempts have all failed. On the other hand, the so called "waxy" starches, whether obtained from maize or sorghum, can readily be flaked by the usual procedures, and, except for minor differences in flavor, the prepared products are not easily differentiated from those produced from *Cassava* starch.

TABLE XXXIV

Cassava Products

Kind	Source	Origin	Water	Protein	Fat	N-free extract	Starch	Sugars	Fiber	Ash
Root	Thorpe	?	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
"	"	?	70	1.1	0.41		21	5	1.1	0.5
Meal	Winton	Africa	11.4	2.1	1.1	82.1	18-24	3-6	1.8	1.6
"	"	Guatemala	12.2	1.8	0.9	78.1	65.8		4.2	2.8
"	"	Dutch		1.8	0.6	80.1				
Starch	"	"Foreign"	12.8	Trace	0.2	86.9			0.08	0.04
"	"	French	16.0	0.45	0.15	83.0			0.00	0.45
"	Sherman	Average of 7	11.4	0.4	0.1		88.0*		0.1	0.1

* Total carbohydrates.

As mentioned already, there are two types of dessert products requiring two different types of starch. These are well typified by the *blanc mange* corn starch pudding and the equally well known tapioca pudding. For special dishes some prefer potato starch to corn starch, as for example in the Danish *rödgröd*, and likewise a modification of the tapioca type starch, sago, is used as a substitute for that product in certain dishes. Price is of paramount importance when a starch is selected for an industrial purpose, but use rather than price is the determining influence when it comes to the housewife's choice between corn and tapioca.

It has long been known that certain so called "glutenous" or "waxy" varieties of grain sorghums, maize, barley, and rice contain a starch having characteristics similar to *Cassava* starch, but this was of purely academic interest until the Japanese attack on the Dutch East Indies suddenly stopped all tapioca importations from the East Indies. This sudden curtailment of available tapioca supplies made substitute starches of domestic origin essential.

Immediately agronomists focused their attention on the varieties of cereals with waxy starch. New sorghum hybrids were produced in Kansas, Texas, and Nebraska, and waxy maize acreage was rapidly increased in Iowa. It now appears that the immediate future will see imported tapioca almost, if not completely, replaced by starch from the waxy sorghums and waxy maize. Whether these starches can hold their own against postwar competition from imported tapioca remains to be seen. However, the domestic product does produce a pudding indistinguishable from that obtained from the imported product. This war-born farm crop constitutes a new industry which may need protection for a few years but which will, with a little encouragement, provide a new and substantial source of farm income. The waxy maize yields about $1\frac{1}{2}$ tons of starch per acre, while the sorghums may produce from $\frac{1}{2}$ to $1\frac{1}{2}$ tons of starch per acre, depending on the variety and the locality.

In 1937 the combined sago, potato, arrowroot, wheat, and rice starch used in the food industries amounted to only a little over 10 million lbs., of which sago represented about 40%. Inasmuch as most of these starches represent at best possible substitutes for either corn or tapioca, without any outstanding or special individual characteristics to recommend them for food uses, their utilization for food purposes becomes a matter of whether they are able to obtain distribution and to compete with corn starch on a price basis. Owing to the large year to year fluctuations in the availability of potatoes, there seems little likelihood of the potato becoming a major source of food starch in the United States. The fact that wheat starch, however, is produced as the by-product of an increasingly important flavor industry based on the conversion of wheat gluten to sodium glutamate and related products places this starch in a better competitive standing.

As the use of starch and starch products is of a very diversified nature in respect to foodstuffs, no attempt will be made comprehensively to discuss each particular application. In the following sections several of the representative uses of both starch and flours will be outlined. The reader is referred to another chapter in this text for a discussion of the utilization of starch products in brewing.

2. Flour in Bread Making. Although starch is the major constituent of flours, other constituents of the grain are also present, in smaller amounts, which enhance its use for some purposes. Wheat flour, with which this discussion is chiefly concerned, contains about 72 to 75 % of starch, 7 to 13% of protein, 0.4 to 0.7% of ash, and small amounts of natural sugars, fats, enzymes, and other organic components. Although the amount of protein present is relatively small compared to the amount of starch present, it exerts a very pronounced influence on the character of products made from flour. Indeed, the art of baking breadstuffs depends to a large measure on the selection of flour with the proper gluten characteristics and the correct transformation and utilization of this constituent before and during bread making.

The ability of flour to swell in cold water or milk and form a dough depends principally on the gluten present. Starch does not swell in cold water (except

to a very insignificant extent and except for a few granules which may have been injured in the milling process). The swelling and water absorption which occur when pure starch is stirred in water at the temperature of the mixing of dough and fermentation result in a granular, non-coherent mass that in no way resembles the plastic dough that results when flour is hydrated. Obviously, starch may be made to swell in cold water and to absorb more moisture at lower temperatures by severe overgrinding and thus materially increase the capacity of a flour to hydrate. This special phase of the subject has received due attention not only in respect to the hydration of flour but as a means of making the raw starch more available for enzymic activity in the mixing and fermentation operations.

Leavening of dough is generally brought about by carbon dioxide gas generated in the dough either by an induced fermentation or by the admixture of carbonates which will release carbon dioxide. The discussion in this section is concerned chiefly with some of the problems involved when the former method of leavening is used.

Fermentation in dough is brought about by the action of enzymes in the flour and by those of added yeast (and occasionally malt) on the carbohydrates and proteins in the dough. Flour contains a saccharogenic diastase, β -amylase, and a fairly potent proteinase called papainase, and yeast furnishes maltase and zymase. The latter are involved in the later stages of fermentation in the production of carbon dioxide, alcohol, and characteristic flavoring compounds. Saccharogenic activity is influenced by the amount of soluble carbohydrate present, the susceptibility of the starch to enzymic action, and the amount of active enzyme available. Often these factors are inadequate for a flour to supply the yeast with sufficient sugar for a normal fermentation. To correct this, such adjuncts may be added as malt preparations, sirups, and sugars. The action of the proteinase must be controlled, for its action, if permitted, produces undesirable results in the baking quality of the dough. Enzymes of the papain type are readily inactivated by oxidation. Hence a pretreatment of the flour, as by added oxidants, atmospheric oxidation during storage, or the addition of oxidizing reagents to the dough, will inactivate this enzyme. Finally, the metabolic state of the yeast employed is also important (1).

The baking properties of flour are expressed in terms of the behavior of the dough in the bakery. Such characteristics are as follows: strength, stability, fermentation tolerance, tolerance to mechanical treatment, and tolerance to oxidizing agents. These have been ably discussed by Blish (2). Strength is the capacity for making a loaf of large volume per unit weight and is principally a function of the protein present. Stability is the resistance a dough offers to becoming tacky or sticky during fermentation. Fermentation tolerance is related to the length of the time interval over which good bread can be kept in a state of fermentation and is dependent on the "gassing power" of the dough. Mechanical tolerance indicates the degree of stability of the dough in mixing and kneading operations. Tolerance to oxidizing agents, when they are added, is a

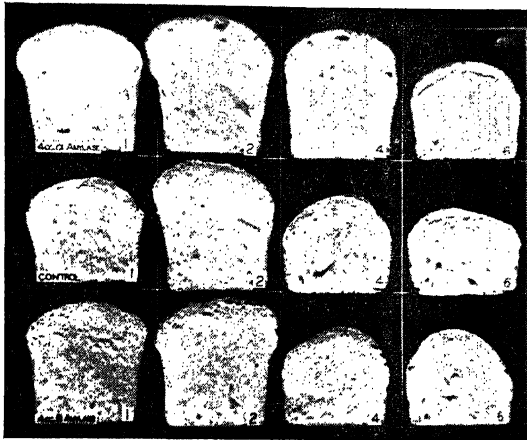
very important property. Differences in the responses shown by different flours necessitate careful control in the use of oxidizing agents by the miller or by the baker in order to secure uniformity in the final baked product. The quality of the baked bread is measured by its volume per unit weight, its texture, its moisture content, the character of its crust, and its keeping quality.

The so called "diastatic power" of a flour, that is its ability to furnish the added yeast with fermentable carbohydrate, depends upon (a) the amylase content of the flour, (b) the nature of the amylases, (c) their availability or activation, and (d) the nature of the original carbohydrate in the flour.

The amylase content of flour varies considerably with the source. Geddes and Eva (3) found that 80 flours varied in diastatic activity from approximately 70 to 300 units. The enzyme constituent present is principally β -amylase and, as is the case in most resting grains, it appears to be intimately bound with other constituents and is not readily liberated. Although dilute salt solutions are effective in releasing the inactive or bound enzyme in wheat, as has been shown by Sandstedt, Blish, Mecham, and Bode (4), it is not likely that the amounts present in dough are very effective in such liberation. The grain is naturally provided, apparently, with a means for releasing enzyme. Ford and Guthrie (5) showed many years ago that a treatment of barley with the protein-splitting enzyme, papain, is effective in the release of barley amylase. They also called attention to the solubilizing effect of salt. Joza and Gore (6) found that treatment with papain greatly increases the solubility of the amylase in wheat flour. Apparently, the amylase is attached in some manner to the gluten. Although wheat contains a papainase, unfortunately the activity of this protein-splitting enzyme cannot be permitted to exert itself in dough. Therefore, many studies have been made to determine and overcome the shortcomings of wheat flours in respect to their diastase content. Stamberg and Bailey (7) have recently published some interesting results which more or less summarize the problem. These investigators added purified enzyme preparations to flour; the enzymes added were β -amylase, which is present in flour at least in combined form, and α -amylase, which, if present at all, is in a more inactive state than the β component. Their experiments indicate that normal flour does not require the addition of β -amylase, since the flour is not materially improved by such addition. However, the addition of small quantities of α -amylase, the starch-liquefying or dextrinizing component of malt diastase, brought about a marked improvement of the volume of the loaf at various fermentation times for the dough. Some of the results of their baking experiments are shown in Fig. 97. These results support the contention of Blish, Sandstedt, and Kneen (8) that the α -amylase is the more important enzyme and that β -amylase has very little effect on the diastatic activity of dough made from normal flour. Sandstedt, Jolitz, and Blish (9) believe that the improvement effected by adding malt to flour is brought about by the α -amylase content of the malt diastase. Not all flours respond equally well to the added α -amylase. From the view-point of starch chemistry, the above results might be interpreted to mean that the inability of a dough to

supply yeast with sufficient fermentable sugar is not so much due to the fact that a large share of the β -amylase is in an inactive state and that there exists a scarcity of this factor, but rather that it is due to a scarcity of suitable substrate for the β -amylase in normal flour. The presence of a starch-liquefying component is required. The latter is apparently better able to function on the native carbohydrate than is the β -amylase.

The question of injured granules cannot, of course, be overlooked in this discussion. Early workers, for example Brown and Heron (10) and Maquenne (11), observed that mechanically injured starch granules are more readily attacked



Courtesy of Cereal Chemistry.

FIG. 97. Interior of bread made with additions of α - and β -amylase in doughs with 1, 2, 4, and 6 hrs. of fermentation. Upper row, 4 cc. of α -amylase added; middle row, control; lower row, 4 cc. of β -amylase added.

than sound granules. The writer has observed this effect with special reference to starch-liquefying enzymes, using prolonged ball milling of corn starch to effect the mechanical injury. It is obvious, therefore, that a flour so milled as to disorganize the enzyme-resistant orientation of even a small proportion of the starch granules present will exhibit an increased fermentation rate, at least in the initial stages. It is this early stage of dough fermentation on which the focus of attention of most investigators is fixed for perfectly understandable reasons. Hard wheats, which require comparatively strenuous grinding, should therefore possess more injured starch granules and, other things being equal, respond more favorably to the action of added diastase than flour from softer wheats. Collatz (12) found, in accordance with the above theory, that the starch of strong flours appears to be more easily hydrolyzed than that of weaker flours. Alsberg (13) has suggested that flours which are weak in "diastatic power" might be overground to correct this shortcoming. This investigator

also suggested the addition of small quantities of severely overground flours to those with deficient "diastatic power" in order to improve them.

The difference in the response of various flours is probably not so simply explained, however, as indicated above. It is quite likely that the character of the gluten and the physical association of the starch and gluten play an important rôle as well. In soft or "floury" wheats, as in other "floury" grains, much of the starch is free, whereas in hard or "horny" grains, the starch is embedded in a matrix of gluten which condition not only limits the accessibility of the starch to the enzyme, but also tends to change the character of the starch as well.²

An alternate solution to the problem just discussed, obviously, is to furnish the yeast with a ready supply of fermentable carbohydrate. It is apparent, of course, that acceleration of dough fermentation is very desirable from an industrial point of view, since production is speeded up, accordingly. Furnishing an active yeast with a ready supply of fermentable material and thus dispensing with the intermediate phase of diastatic activity would seem to be the logical method of best contributing to this end. Many relatively inexpensive sources of carbohydrate are available in the forms of sirup and sugar. Not all sirups, however, possess high fermentability. Malt sirup does contain a high percentage of fermentable solids and may be added for this purpose. According to Rumsey (14) some 30 million lbs. of malt extract have been used annually by American bakers in the past. But since malt sirup contains a very plentiful supply of diastatic enzymes, as well as available carbohydrate, the danger arises that the dough may be oversupplied with diastase. Stamberg and Bailey (7) point out that sogginess of crumb may result from adding too much diastase to the dough and suggest that many of the poor baking results in the past may be traced to this factor.

The fermentability of the relatively less expensive and as easily handled sirups from corn starch has recently been investigated by Kerr and Schink (15). Some of these types, such as for example the "Corn Sirup, Unmixed" (CSU) of commerce, are rather low in the percentage of solids fermentable by yeast. They contain usually less than 50% of fermentable solids. Kerr and Schink point out that several concerns have developed newer types of corn sirups made by processes involving a diastatic treatment. These sirups are of the order of 65 to 70% fermentable. A sirup such as described in the patent of Dale and Langlois (16) is an example. More recently Kerr, Meisel, and Schink (17) disclosed methods for producing very highly fermentable, stable corn sirups, with fermentable solids in the range of 80 to 85%. The latter should, accordingly, be very near the ultimate for those industries which prefer a readily available source of fermentable carbohydrate in liquid form.

Of course the peak in fermentability is the pure sugar, glucose or dextrose as it is more commonly referred to in industry. Dextrose is theoretically 100% fermentable. Although cane sugar is fermented by yeast, it requires a hydrolysis

² Refer to the sections on corn starch in Chapters I and II for further comparisons of "floury" and "horny" starch.

to its simple sugar constituents, dextrose and fructose by the enzyme invertase before the fermentation reactions, proper, begin. The development of the manufacture of pure, crystalline dextrose in the United States during the last two decades has been a great boon to the baking industry, among others. Its use was readily adopted by the baking trade as an additive to doughs to furnish the yeast with the required amount of fermentable substance to produce an excellent bread; and today the baking trade is one of the largest consumers of this product, derived from starch, the production of which now requires probably a quarter of all the corn starch milled in the United States.

The advantage of dextrose to the baker is not only that it is quickly and completely fermentable but also that it improves the quality of the bread in several respects. The golden brown color of the crust and its keeping quality after baking are two such qualities which may be mentioned, which result from the addition of dextrose in slight excess of the actual requirements of the yeast.

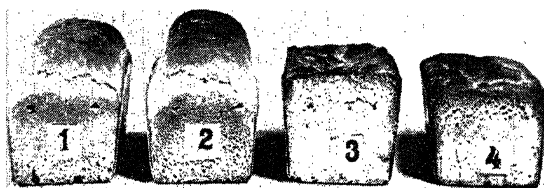
All baking aids or adjuncts are not necessarily concerned with carbohydrate modification or utilization in dough or bread making. Some aids are considered as yeast foods or stimulants, while others are useful in inactivating the proteinase of wheat flour, which enzyme exerts a deleterious effect on the wheat gluten. Balls and Hale (18) and Jorgensen (19) have shown that wheat proteinase is a papain-like enzyme. It is activated by reduced glutathione and is inactivated by oxidizing agents such as are present in some bread improvers. Potassium bromate, for example, inactivates the proteinase of wheat flour. These enzymes act first to produce a clot with the flour proteins and then to produce a liquefying action so that the dough loses its strength or ability to retain the carbon dioxide of fermentation. Little or no raising of the dough results in extreme cases.

Balls and Hale (20) carried out baking experiments in which potassium persulfate was added to inactivate the proteinase. Similar loaves were made which included papain and glutathione instead of the persulfate. The results of these baking tests are shown in Figs. 98 and 99. The photographs of the baked loaves clearly illustrate the value of destroying the proteinase and the adverse results obtained when the papain activity is increased.

The aging of flour, either naturally or as induced by chemicals, has long been known to improve its strength. It now appears that the action is not so much a change in the physical properties of the gluten or the starch, as it is rather, the increased strength which results from such action due to a reduction in the proteinase activity of the flour, as was further demonstrated in the experiments reported by Kent-Jones (21).

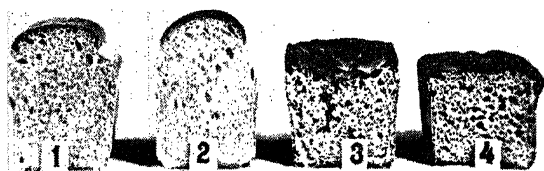
For a full description of baking tests and for a discussion of various factors and variables which influence the results, the treatment of which is outside the scope of this chapter, reference is made to the reports of Blish (22), Markley and Bailey (23), Geddes, Larmour, and Mangels (24), Schultz and Landis (25), Landis and Frey (1), and the report of Kent-Jones mentioned above. Reference is also made to a recent report of Swanson and Johnson (26) relating to the use of a recording dough mixer in the study of the quality of flour for baking.

An important factor to be considered in bread is its keeping quality after baking. Carbohydrate chemists have frequently sought to show a relationship between the modification of the starch in bread making and the staling of bread which follows. The many researches on the subject by Katz and coworkers have been summarized by Katz (27) and therefore only the salient points of his work will be reviewed. The exact interpretation of his theories has been changed by some and these theories have been questioned by others. The essence of



Courtesy of Cereal Chemistry.

FIG. 98. Exterior of 1 lb. loaves of bread containing: Loaf 1, no addition (control); Loaf 2, 50 mg. of potassium persulfate; Loaf 3, 50 mg. of papain; Loaf 4, 50 mg. of glutathione.



Courtesy of Cereal Chemistry.

FIG. 99. Interior of loaves of bread shown in Fig. 98.

what Katz sought to confirm, that the change which occurs within a partially gelatinized starch granule as it cools and ages is closely associated with a change in water relationships and is an essential part of the staling process, still remains to be conclusively disproved by experiment.

The obvious change in bread as it stales is that its texture changes from soft or tender to a hard, granular state. The bread loses its characteristic cohesiveness and becomes crumbly. Katz confirmed the reports of early workers and showed that this change was not merely one of dehydration. In a preliminary examination of the problem of staling, Katz and Verschaffelt concluded that staling was principally the result of a change in the physical properties of the gelatinized or

partly gelatinized starch in the bread. As the change was examined with the aid of the microscope, it was seen that the starch granules became more sharply outlined. Upon closer examination it was found that the granules became doubly contoured and that between the two was what appeared to be a layer of air. Katz then took as criteria for staling (*a*) changes in the hardness of crumb, which was tested with a specially designed penetrometer, (*b*) changes in the swelling power of the crumb, determined by noting the apparent volume of 10 g. of pulverized bread-crumbs when shaken and allowed to stand from 24 to 48 hrs. under an excess of water, (*c*) changes in the amount of soluble material made by extracting a known weight of bread with cold water, and then filtering and precipitating the solids from the concentrated extract with an excess of alcohol.

Applying these tests to fresh bread and to the same bread 48 hrs. after baking, Katz found the following representative figures.

	Hardness	Swelling power cc.	Soluble per cent
Fresh bread	0.240	52	5.39
Stale "	0.030	34	2.88

Using the above criteria, Katz attempted to demonstrate that staling is a process induced by a change in temperature. He found that bread kept 48 hrs. at 60° C. is still fresh by these same tests, whereas bread kept at 0° C. is more stale than when kept at room temperature. But, on the other hand, greatly reduced temperature, -185° C., prevented staling entirely. The point of maximum velocity for staling was found to be -2° to -3° C. A close parallelism is therefore apparent between these results and the effect of temperature on the speed of retrogradation as observed by Maquenne in his classical researches on the subject.

As might be anticipated from the theory, the addition of alkaline substances, such as pyridine and dipropylamine, was found to be effective in the prevention of staling. However, if staling is due to cross-bonding of molecules of starch by the elimination of water, as Katz suggested (or as the modern theory would explain it, by the formation of hydrogen bonds between carbohydrate hydroxyls, with the release of associated water molecules), it is surprising that acids, chloroform, and alkyl halides should have been reported to be without effect. The latter compounds, it would seem, should have accelerated staling, on the assumption that staling involves an orientation of the starch molecules.

Katz then showed that the changes which take place when bread stales are in the same direction as when starch alone is gelatinized at the same starch to water ratio as in bread and when heated to the same temperature, on the average, as that reached within the loaf of bread when it is baked.

Opposed to the view that staling is essentially a retrogradation effect in the starch, three arguments, mainly, have been advanced by more recent workers. It is pointed out that, whereas stale bread is considered more digestible than fresh bread, retrograded starch is less readily attacked by diastase than a freshly cooked starch paste. This argument is based on two false premises. The

principal premise should have been stated, that fresh bread is more "indigestible" than stale. This is due to the doughy or soggy character which fresh bread assumes when it becomes moist, as in mastication. This physical condition prevents the ready absorption of enzymes from the saliva and more particularly of the gastric juices. These chunks, therefore, must remain longer in the stomach and induce the effects commonly associated with indigestion. Secondly, the assumed premise is made that the enzymes responsible for the digestion of bread in the stomach and the liquefaction of starch *in vitro* are the same. This is not true. Proteolytic enzymes are concerned principally in action in the stomach. If the bread is digested, it is converted into a slurry due to a liquefaction of the protein, the mass no longer being held together by a binding agent, the protein. The reaction here is acid, and diastases are sensitive to acidity. This is particularly true of starch-liquefying enzymes, and exposure to a slight acidity may be used as a means of "purifying" β -amylase preparations.

It is quite probable that unless the salivary amylase is enclosed in and protected by a more or less impervious mass such as is the case when fresh bread is eaten, very little diastatic activity survives in the stomach.

It is also pointed out that stale bread may be freshened by a moderate reheating, whereas retrograded starch is not redispersed by heating to the same temperature. The argument is based on the observation that, if starch is thoroughly gelatinized and diluted, or *vice versa*, so that the starch is thoroughly dispersed to allow the more linear chains of starch molecules to orient and precipitate, a granular product is formed which cannot be redispersed except at very high temperatures. In bread, however, there is not sufficient water for complete dispersion or even complete gelatinization of the starch. Indeed, Katz pointed out that the water present is sufficient only to permit the initial stages of gelatinization. Starch heated with a corresponding proportion of water merely swells to form a salvy gel in the case of the cereal starches. Quite likely the formation of such a gel involves some orientation or association but it is definitely more complex, involving, no doubt, the more complex configurations within the starch as well as the linear chains. Cross-bonding probably takes place at random points. But it is hardly comparable to the more complete, "two dimensional" orientation of linear chains with each other, which follows when starch is completely dispersed in dilute solution. Although it is possible that the retrogradation effects in these partially gelatinized starch gels cannot be completely reversed by heating to temperatures under 100° C., these gels can be reconstituted into pastes by adding the required water and then heating and stirring. It has not been proved, moreover, that stale bread refreshed by heating is identical physicochemically with freshly baked bread.

Finally, it is often pointed out that the retrogradation of starch in a paste proceeds more slowly than the staling of bread. However, considering the variety of substances known to speed retrogradation effects and the fact that bread dough contains several of these, such as fats and calcium salts from added milk, this argument does not seem important.

On the other hand, strong evidence that orientation in starch is concerned with staling follows from the observation of Katz that acetaldehyde and propionaldehyde will prevent or greatly retard staling. Although the action of aldehydes on starch is admittedly complex, it seems quite certain that in the early stages of this action a loose bond is formed with the carbohydrate through hydroxyl groups. The starch becomes more difficult to hydrate and requires substantially higher temperatures and higher concentrations of alkali to disperse or dissolve it. If untreated starch pastes are allowed to age, there is a gradual reduction in the intensity of the blue color with iodine as the retrogradation process is in progress; that is, the starch molecules gradually orient with themselves and become less able to orient with iodine molecules. The presence of a small amount of acetaldehyde in the starch paste, however, will prevent the loss in the ability of the paste to form a blue color with the iodine. Also, if the acetaldehyde is removed, the normal decrease in intensity of the blue iodine coloration begins at once. Similarly, bread may be kept fresh by added acetaldehyde and if the acetaldehyde is removed, by passage of a current of moist air through the bread, the staling process starts at once and follows its normal course.

The above facts are explained logically on the assumption that the association of the starch molecules with aldehyde and with each other is of the same type and through similar groups in the starch molecules, which groups are very likely the hydroxyl groups. The association of the starch molecules with iodine, however, is of a different type, as discussed elsewhere in this volume.³ The latter is essentially a sorption effect in which the starch chains coil around the iodine molecules. Hence, whereas acetaldehyde can prevent hydroxyl association by a blocking effect, it does not prevent the chains from forming coils. But removal of the aldehyde, however, allows cross-bonding and obviously the formation of coils by each separate chain becomes more difficult. It is logical to suppose that the aldehyde tends to act on the starch in bread in a similar fashion, and in consequence orientation by cross-bonding is made more difficult and staling is prevented until the blocking influence is removed.

In conclusion, although staling may not be due exclusively to retrogradation, as the term is used in the more usual sense to denote the less reversible orientation of the linear molecules, it can be concluded that a related change in the starch, hydrogen bonding of hydroxyl groups, both on linear and branched configurations, with the probable elimination of 1 or more water molecules per bond formed, is the principal action in staling. Quite naturally, as suggested by Alsberg and others, the change in the physical state of the gluten as the bread cools and ages cannot be entirely eliminated from an explanation of the total effect observed.

3. Crackers and Biscuits. Starches are used in the biscuit industry for two purposes: as an aid to manufacturing and as a biscuit ingredient. When powdered sugar is not used soon after grinding, it is common practice to add 3% of redried corn starch to prevent lumping. Corn starch is generally employed for dusting the canvas aprons on icing machines to prevent sticking. Starch would

³ See Chapter XVII.

be an ideal agent for dusting some biscuit doughs during machining were it not for the fact that the starch "bridges" and hence does not feed evenly in the mechanical spreading and shaking devices. Recent advances in producing a free-running starch should eliminate this difficulty.

Perhaps the oldest use of starches as a biscuit and cracker ingredient is that of arrowroot starch or "flour" in the widely produced arrowroot cracker or "hard-sweet." Arrowroot biscuits, because of their starch content, have been regarded as the most suitable of all biscuits for children, infants, and invalids, and arrowroot starch was for long considered to have almost a medicinal value. The reasons for this high esteem, in the opinion of the author, are (1) it was one of the first relatively pure starches of commerce to be produced; (2) the price of the starch always has been comparatively high; (3) it originates from the semi-tropics; and (4) early publications (28) indicated that arrowroot starch is the most easily saccharified of all starches by the saliva.

The arrowroot cracker was and still is a most nutritious and easily digested baked product as the following old standard formula indicates. However, inspection of the table of ingredients shows that these qualities can hardly be due solely to the presence of the arrowroot starch.

English Arrowroot, Hard-Sweet

200 lbs. soft flour*	2 lbs. salt
25 " arrowroot flour	4 " cream of tartar
20 " invert sirup	2 " soda
45 " butter	vanilla to suit
65 " powdered sugar	20 " whole sweet milk (variable)
20 " eggs	

Method—Cream arrowroot, shortening, sugar, eggs; add vanilla and salt. Sift soda in flour and add. Put sirup on top of flour with milk and make semisoft, smooth, plastic dough. Break down and let stand 2 hrs. Rebreak and roll well. Run medium thin. Bake on wires.

The chief ingredient of most biscuits and crackers is wheat flour of which about 80% is wheat starch. Yet no attention has been paid directly to the rôle of wheat starch in the industry. The wheat starch becomes important by difference, so to speak, because it is the principal ingredient the flour contains in addition to protein. Biscuit flours are "soft" flours and have a protein content, roughly, of 7.0 to 8.5% for sweet goods and cracker doughs and of 8.4 to 10% for cracker sponges.

In order to give desirable eating qualities to the baked products as well as to avoid the use of an exceptionally high proportion of sugar and shortening which would otherwise be required, it is necessary to keep the protein of the flour sufficiently diluted either with the natural wheat starch or with some added starch such as corn or tapioca. Flours which contain less than 7.0% of protein (approximately, but the amount varies with the flour) exhibit most of the char-

* In this and the following formulae, flour denotes wheat flour unless specified to the contrary.

acteristics of a starch paste when they are used for baking, while those which contain a higher protein content exhibit more of the characteristics of a "dough."

It is desirable for production of sweet goods to keep the flour as near to this critical composition as possible. Because of the differences in the annual crop, prices, etc., such flour is often not available. It becomes necessary then either to increase the sugar and shortening content of the biscuit or to dilute the protein with some other ingredient. Corn starch is usually selected for this latter purpose. During World War I, good biscuits were made with flour diluted with as much as 50% of corn starch. This, however, is more than the optimum dilution. By using 5 to 20% of starch (based on flour weight), depending on the protein content of the flour, a 15% saving in the amount of sugar and shortening results. In "honey" doughs it is possible to eliminate the shortening entirely. In a soft batter dough, such as the vanilla wafer, a 12.5% reduction in the amount of sugar and shortening can be effected. Furthermore, the wafer dough does not tend to stiffen and to become tough during the time after it leaves the mixer until it is through machining. The baked product is also more tender than biscuits which are made without starch.

Both raw and gelatinized corn starch are used in the manufacture of crackers. When the flour is of normal strength, the addition of 10% of corn starch will save an equivalent amount of fat and 15% of the yeast and at the same time the starch addition produces a more tender cracker. When the flour is very "strong," "bucky," and generally hard to handle, the addition of about 5 to 10% of a gelatinized corn starch product ⁴ with a cold water absorption of about 10 : 1 will enhance fermentation, give a smooth running dough, and result in the production of a cracker which does not buckle or "cup" during the baking processes.

The advantages of using corn starch for baking biscuits and crackers may be summarized as follows: (1) the flavor is improved; (2) greater effectiveness of the added flavors is secured; (3) the interior color of the biscuit is clearer; (4) there is a saving in the amount of shortening required; (5) saving of sugars; (6) saving of yeast (crackers); (7) there is a reduction of sticking of finished goods (this is especially true of "honey" goods in warm weather); (8) the grain or texture of the baked product is improved; (9) a much better bottom face is produced on the cookies.

In bake shop specialties, such as the ice cream cone or the sugar wafer shell, the character of the flour is even more important than in regular sweet goods and crackers. A flour that is too "soft" will make a product which will crumble easily, while a flour which is too "strong" will produce a brittle shell. In this case, also, the proper flour is rarely available or the cost makes its use prohibitive. Hence, both corn and tapioca starch are used by the industry to modify the

⁴ Examples of the type of product indicated are those such as are sold under the trade names "Amijel" and "Amidex."

characteristics of the flour. Tapioca has been preferred to corn starch because tapioca appears to give a more tender cone or shell which is less brittle, and consequently less breakage of the product in packaging and shipping results. A better flavored shell may be made when tapioca starch is used. Lastly (probably the most important reason of all), the price of tapioca has been consistently lower than that of corn starch. As high as 40 to 50% of starch (based on flour) may be used to make these bake shop specialties. The average amount of starch used, however, is probably 5 to 7%, and this value rarely exceeds 15%.

Occasionally potato starch and flour are used to produce a specialty product, although their use is not common in the biscuit and cracker industry. The accompanying formula is an illustrative example of a potato cracker.

Potato Cracker

100 lbs. flour	2 lbs. salt
40 " potato flour	5 " cheese
20 " " starch	16 " butter
40 " boiled potatoes	20 " water

Directions—Put potato flour, starch, and boiled potatoes in the mixer; mix for 5 min. Add water, salt, cheese, and butter; mix for 5 min. Add flour and mix clear. Top with fine salt. Spray with coconut oil after baking.

Because of the low moisture content (4 to 6%) of finished biscuits and crackers, the retrogradation of starch is not a factor in their staling, except when they are allowed to absorb abnormal amounts of water. The keeping quality of the shortening is usually the limiting factor.

The accompanying general skeleton formulae illustrate how corn starch is used in standard biscuit and cracker products. For the purpose of simplicity all formulae are based upon 100 parts of flour. In practice, the size of the batch of dough varies with the capacity of the essential equipment for handling it. The ability of the dough to withstand aging is also a factor which limits the size of the batch.

Soda Crackers (Peeling Machine)

Sponge:

80 lbs. flour	5 lbs. gelatinized starch
$\frac{1}{4}$ lb. yeast	32 " water
$\frac{1}{2}$ " dextrose	

Preferred Mixing Order and Method—Yeast, water, yeast foods, part flour, starch; ferment. Set at 72° F., out at 82° F. after 18 hrs.

Dough:

20 lbs. flour	2 lbs. water
12 $\frac{1}{2}$ " shortening	$\frac{1}{2}$ lb. soda
1 lb. salt	

Preferred Mixing Order and Method—Add all ingredients; ferment. Out at 84° F. after 5 hrs.

Graham Crackers (Cutting Machine)

75 lbs. flour	5 lbs. invert sirup
25 " graham flour	1 lb. salt
10 " starch	1 " soda
12 " shortening	$\frac{1}{4}$ " ammonium bicarbonate
13 " sugar	22 lbs. water
13 " dextrose	

Preferred Method—Make a hot sirup (200° F.) of all sugars, salt, and water, and add to all other ingredients in the mixer. Bring dough out at 125° F. Run before dough cools off.

Honey Type (Cutting Machine)

100 lbs. flour	0-6 lbs. shortening
20 " starch	2 " soda
75 " sirup (invert, honey, dextrose, and molasses) 80% solution	14 " water

Preferred Method—Mix all ingredients except flour and starch. Add starch and flour.

Semihard Sweet (Cutting Machine)

100 lbs. flour	16 lbs. water
5-10 " starch	3 " eggs
28 " sugar	$\frac{1}{2}$ lb. soda
12 " dextrose	$\frac{1}{4}$ " cream of tartar
1 lb. salt	0-5 lbs. shortening

Preferred Method—Cream sugar and shortening; add eggs; add water; add flour and starch.

Fig Bar Dough (Special Machine)

100 lbs. flour	8 lbs. eggs
5 " starch	3 " dry skim milk
30 " sugar	20 " water
12 $\frac{1}{2}$ " dextrose	$\frac{1}{2}$ lb. soda
2 $\frac{1}{2}$ " invert sirup	$\frac{1}{4}$ " cream of tartar
20 " shortening	1 " salt

Preferred Method—Cream sugar and shortening; add sirup; add eggs; add water; add starch and flour.

Honey Dough (Wire Cut Machine)

100 lbs. flour	1 lb. salt
10-20 " starch	1 " soda
70 " sirup (honey, invert, dextrose, molasses)	$\frac{1}{2}$ " ammonium bicarbonate
15 " sugar	$\frac{1}{2}$ " cream of tartar
0-5 " shortening	22 lbs. water

Preferred Method—Mix flour, starch, sugar, and other ingredients. Thin down with water slowly.

Wafer Dough (Wire Cut Machine)

100 lbs. flour	10 lbs. eggs
20 " starch	1 lb. soda
50 " sugar	$\frac{1}{2}$ " cream of tartar
17 " dextrose	$\frac{1}{2}$ " ammonium bicarbonate
33 " shortening	1 " salt
5 " dry skim milk	65 lbs. water

Preferred Method—Cream sugar and shortening. Add eggs; add water; add starch and flour.

Scotch Shortbread (Rotary Machine)

100 lbs. flour	10 lbs. water
10 " starch	$\frac{1}{2}$ lb. soda
20 " sugar	$\frac{1}{2}$ " cream of tartar
15 " dextrose	35 lbs. butter and (or) shortening
10 " eggs	

Preferred Method—All ingredients in mixer at once give good results.

Sugar Wafer Shell (Special Machine)

(Cones are similar.)

100 lbs. flour	$\frac{1}{2}$ lb. soda
7 " starch	$\frac{3}{4}$ " salt
2 " shortening	$\frac{1}{4}$ " ammonium bicarbonate
75 " water	1 $\frac{1}{2}$ lbs. egg yolk
75 " milk	

Preferred Method—Mix all ingredients together at once except the water and milk. Thin down gradually with these liquids.

Tapioca and corn starches were once considered important ingredients for use in the biscuit marshmallow process. The following skeleton formula is typical.

21 lbs. water	1.6 lbs. <i>Cassava</i> starch
31 " corn sirup	2.4 " gelatin
44 " sugar (partially inverted)	

Preferred Method—Suspend the starch in part of the water. Add the sugars. Heat to 200° F.; add the corn sirup. Add gelatin which has been dissolved in part of the water. Cool to room temperature and beat in convenient batches.

As the art of compounding biscuit marshmallow progressed, the starch was withdrawn from the formula. The consensus of opinion among biscuit bakers has been that the starch has a tendency to hold down the volume of the marshmallow. It does, however, contribute a certain degree of "set," or stability, to the marshmallow, which characteristic is particularly useful in summer weather.

Lately, the practice of adding 0.25 to 0.50% of a powdered, gelatinized corn starch product (cold water absorption about 10 : 1) at the beater has received favorable attention. The added starch product permits the marshmallow to carry 1 to 2% more water, which promotes tenderness. At the same time the

starch gel increases the stability of the marshmallow for machining, packaging, and shipping.

Preferred Method and Typical Seasonal Formulae for Biscuit Marshmallow

Stock sirup	Winter lbs.	Spring and fall lbs.	Summer lbs.
Sugar	5.0	10.0	20.0
Dextrose	5.0	10.0	12.0
Corn sirup	25.0	27.0	30.0
Invert sirup	55.0	36.0	17.5
Water	8.2	14.8	17.0
Gelatin (175 bloom)	1.5	1.75	2.0
Gelatinized starch	0.3	0.40	0.5

Method—Make a sirup of the sugars, sirups, and part of the water. Dissolve the gelatin in part of the water. Add the gelatinized starch and the gelatin solution at the beater.

Corn or tapioca starch is sometimes used to break up the excessively hard, crystalline structure of flat and “trolley” icings. These icings form a thin, semitransparent coating of small sugar crystals which are held together by a small amount of sirup. Illustrative formulae are given.

Flat Icing

26.5 lbs. water	0.9 lb. gelatin
47.0 “ sugar	5.0 lbs. powdered starch
5.6 “ corn sirup	15.0 “ “ sugar

Method—Dissolve the sugar and corn sirup in part of the water at 200° F. Add the gelatin dissolved in part of the water. Beat to 150° F.; add the powdered sugar and starch, and then beat to the proper consistency.

Trolley Icing

20 lbs. water	72 lbs. sugar
2 “ corn sirup	6 “ starch, powdered

Method—Mix the ingredients and keep at 105–110° F., stirring continuously.

4. Salad Dressings. During the last 10 yrs. and particularly during the last 5, an extensive market has been opened to starch with the development of new formulations for salad dressings. The most important of these are of the mayonnaise type. Mayonnaise consists essentially of a vegetable oil emulsified with egg albumin, vinegar, mustard, salt, and other less important condiments. The newer salad dressings differ essentially in the fact that the vegetable oil is emulsified with a relatively large proportion of gelatinized starch and less or, in the extreme case, no egg albumin. The result is also that the percentage of oil in the dressing is appreciably reduced. The newer type of salad dressing may therefore be made with considerably less cost, since the price of both vegetable oil and eggs is decidedly more than that of starch.

The newer dressing is preferred for dietary reasons in some cases because of this alteration in composition. No intimation is intended that the nutritional value of starch is superior to that of proteins and fats. All three types of foods

have a very proper place in a well balanced diet. Carbohydrates furnish energy and body warmth as do fats. The latter, however, after saponification, may be more readily stored as fat tissue, if there is no immediate need for energy. The utilization of fats for energy depends upon carbohydrate utilization. In simple words, fats may be said to burn in the flame of the carbohydrates. The presence of substantial quantities of available carbohydrates, therefore, facilitates the utilization of fats, especially when large quantities of the latter are eaten, as for example in a rich salad dressing. Proteins, on the other hand, are a source of building material for muscle and other tissues. This is to replace normal wear and tear in adults and to facilitate growth in children. An overabundance of protein, so that the system is forced to call on protein for energy requirements to a large extent, places an undue strain on the deamination and other nitrogen-converting functions of the liver and overtaxes the kidneys to eliminate excesses of these nitrogenous bodies. The normal adult, particularly one who is inclined to obesity, might find the newer salad dressings less conducive to an increase in body weight than the former type. The active person will secure a more balanced composition to utilize for energy requirements.

The use of starch in this field depends upon its emulsifying power, as stated above. Starches are, however, rather poor emulsifying agents; that is, the emulsifying power of a gram of starch is much less than that of a gram of a standard emulsifying agent, such as soap. Stable emulsions may be made with gelatinized starch, but they require a relatively large proportion of starch and special methods to accomplish the result. The use of starch to emulsify vegetable oils has been reviewed recently by Goikhman (29, 30).

The use of a relatively large proportion of starch to produce a stable emulsion with oil in salad dressings requires that another characteristic be imposed on the starch intended for this use. The gelatinized starch added must not change the texture or other characteristics of the salad dressing which the consumer has become accustomed to accept as standard. Some starches tend to gel; some tend to develop a "short" consistency. Furthermore, some starches will resist the tendency to set to a firm gel because of the oil present. On the other hand, in such a medium they will show a pronounced tendency to retain less water. A syneresis develops, and the salad dressing begins to separate a water phase.

The gelatinized starch must not be adversely affected by the action of other ingredients present. Salad dressings are normally quite acid because of the added vinegar or acetic acid. Some starch pastes are very materially thinned in the presence of as weak an acid as acetic. In this case, the decrease in viscosity also is paralleled by a decrease in the emulsifying power. The result is first an undesirably thin salad dressing, and later an unstable one. Some starches are more susceptible to a physical breakdown when cooked with sugar than are others, and many formulae for the newer salad dressings contain substantial amounts of sugar.

Very few starches indeed meet all the requirements already listed plus those imposed by the manufacturing operation to be described. Normally, therefore,

a combination of starches is selected, two or more types being used to impart the desired characteristics to the final gelatinized mixture. Corn starch pastes have more body when cooled to room temperature than the more common non-cereal starches, but they are inclined to be of "short" consistency or, if unmodified, to gel. Corn starches are, however, fairly stable in the presence of acid such as acetic and, once the gel structure has been broken down, the remaining body is fairly stable in the remainder of the emulsifying operation. When they are unmodified, their water retention is not as good as that of some of the other types of starch. Tapioca and some of the other non-cereal starch pastes are softer and are fairly good emulsifying agents but, by themselves, produce a body which is too fluid or mobile. They are weaker in the presence of acetic acid and when cooked with sugar. A common practice is to use a combination of some corn starch and some tapioca.

Nearly each manufacturer has formed his own idea of the proportions of each starch to use, and these formulations show a wide variation. Surprisingly enough, however, the end-product in each case varies little in major characteristics from those produced by use of other formulations. The explanation is a relatively simple one. During the development of the newer salad dressings, few modifications of either corn or tapioca starches were available for this purpose. Indeed, in many cases, the maker of salad dressing used unmodified starches of both types. He installed the type of emulsifying or homogenizing equipment which was deemed to be most suitable for his purpose and experimented with different combinations of corn and tapioca starches until the desired result was obtained.

Emulsifying equipment varies in design from that at one extreme, which is little more than a beater, to the other extreme type, which may be a high pressure homogenizer. In between is the colloid mill type of emulsifier. The proportion of corn starch or modified corn starch to tapioca which is found to produce the desired body and other characteristics in a beater type of emulsifier quite obviously would not be expected to produce the same characteristics in a colloid mill type. Nor would a proportion of a given modified corn starch be expected to be interchangeable with a like proportion of another modification, when the same type of equipment is used. Formulae must therefore be varied to suit the type of equipment installed by each manufacturer of salad dressing.

The attempt of the starch manufacturer to introduce one particular modified starch, *e.g.* a corn starch, to meet all requirements met with some difficulty. The result is that several types are marketed to suit varied conditions. Attempts to use these types interchangeably has frequently led to disappointing results for the maker of salad dressing.

Formulations will be found to vary for another reason. Salad dressings are of two general types which may be divided on the basis of oil content, a salad dressing of high oil or of low oil content. Some manufacturers market both types. With most equipment used, the ratio of the heavier bodied corn starch is increased in salad dressing of the high oil type.

An example of the manufacturing process, as it relates to the utilization of the starch, is as follows: The following ingredients are mixed in the proportions, by weight: corn starch, 6; tapioca, 4; acid (100 grain vinegar), 1; mustard, 1; salt, 1; sugar, 20; water, 67. The mixture is cooked in a closed, jacketed kettle for 30 min. at 180° F. The paste is dropped into a cooler, where it is reduced in temperature of 90° F. and then poured into containers for storage. About 18 to 24 hrs. at 50° F. are sufficient to complete the primary setting of the paste. After the storage period, the paste is added to the oil and egg, in the emulsifier, in the desired proportions. This paste has been used in the thick salad dressings of high oil content. The ratio of paste to oil, however, will be accurately adjusted, depending on (a) the ratio of egg to oil used and (b) the homogenizing equipment available for use.

The ratio of tapioca to unmodified corn starch in the mixtures used varies normally between 33% tapioca to 67% corn starch and 67% tapioca to 33% corn starch.

Present research is concerned with the production of a single starch which will avoid the necessity of using mixtures. Several grades will most likely be required to meet the varied manufacturing processes, as stated above.

5. Starch in Baking Powder. Baking powder consists essentially of an acid or acidic substance and a carbonate, both in dry form, which, in the presence of water, react to liberate carbon dioxide gas. When this gas is liberated within a dough, leavening results as the temperature is raised in the baking operation, owing to the thermal expansion of many minute gas pockets which have been trapped by the glutenous portion of the flour. The acidic component, for example calcium acid phosphate, sodium acid tartrate, or sodium aluminum sulfate, is usually such a feeble acid that heat is required to promote its reaction with the carbonate (*e.g.*, sodium bicarbonate). The reaction is appreciable at lower temperatures, however, and indeed some manufacturers claim that their product begins to react as soon as it is stirred into the batter. To maintain the efficiency of a prepared baking powder in storage, therefore, the two opposing ingredients, the acid and the carbonate, should be kept dry and kept from too intimate contact in spite of the fact that both are mixed together as fine powders. Accordingly, an inert material is added which acts as a diluent. The latter should be pure and edible, preferably white, free from characteristic odors, and capable of maintaining a free-flowing, powdery condition under adverse circumstances. Corn starch admirably meets all of these requirements, especially if processed for this use. The manufacture of baking powder represents not only one of the largest outlets for corn starch prepared for food purposes, but also it is one of the largest of applications for which the starch is not utilized for its paste- or sol-forming property.

As also for a related use, the blending of starch with confectioner's sugar, an advantage in the use of starch is that when it is dried to a low moisture content it may act as a moisture "acceptor." That is, when dehydrated to the range of 5% moisture, the starch will, owing to its hygroscopicity, preferentially absorb

and adsorb about 7% or more of its weight of moisture, should the mixture be exposed to a high relative humidity or dampness. At 12% moisture content, the starch still retains a relatively free-flowing condition, and if specially processed, starches result which are extremely free-flowing even at 14 to 15% moisture content. The moisture-binding power of starch is of additional value in baking powder in that it tends to keep the acid and carbonate in a drier state and hence tends to prevent them from losing their leavening efficiency through premature reaction. However, when native starch is dehydrated by redrying, it frequently develops a characteristic odor in storage. In the case of corn starch this is due to the chemical instability of certain non-carbohydrate substances associated with the starch, which condition is promoted by the redrying operation. This characteristic property of starch may be altered by a treatment of the starch before dehydration. Chapter III has dealt with the control of starch odors as well as methods to obtain a white and mobile, powdered starch.

By dry starch "mobility" is meant the freedom to flow particularly through sieves or sifting devices. The rate at which a fixed weight of starch will pass a given mesh sieve, when a limited amount of agitation is used, may be taken as a relative measure of mobility, as described in Chapter VI. This obviously desirable characteristic of a good baking powder starch parallels another property important for this use. Kerr⁵ has observed that the mobility of dry starch is directly proportional to its apparent volume per unit weight; the more free-flowing the starch, the greater the volume occupied by a pound of the starch. In addition to its appeal to consumers who prefer to buy on a volume basis rather than weight, that is, prefer to receive a larger package for a certain price, the property of contributing a greater volume for a definite addition of starch by weight possesses a real merit as well. A greater volume per unit weight in a product containing the powdered starch indicates that there is more free space in this mixture, which in turn allows the conclusion that the ingredients separated by the starch are in less intimate contact. This condition is obviously desirable in respect to baking powder.

One of the most practical ways to secure high mobility in a dry starch is to treat the starch when wet with oxidizing agents under controlled conditions, as indicated by Kerr.⁶ This worker has also patented a process in which oxidizing agents are also used for producing a white starch much less disposed to develop undesirable odors when dehydrated and stored (31).

6. Confections. In addition to the wide-spread use of derived products from starch such as dextrose and confectioners' corn sirup as sweetening agents in confections, starch and modified starches are also used in the manufacture of several types of candies. Dextrose and corn sirup are employed in the manufacture of hard candies, fudge, nougats, marshmallow, gums, fondants, and coatings as well as other types of confections. Starch is used principally in the manufacture of gums, pastes, and panned sweets, either as an ingredient of the

⁵ Unpublished data.

⁶ See Chapter III.

confection or as an aid in manufacture as for the production of molds. The technology of candy manufacture has been given by Jordan (32), Scarborough (33), and others.

A variety of gum confections are produced by the use of starch. The nature of the product depends not only on the formulation used but also on the cooking and finishing procedures. The formulation may be varied by the addition of colloids other than starch, such as gelatin and gum arabic, or by the choice of the starch employed. The most popular gum in America is made from a starch jelly to which no other colloid is added, and when a thin boiling starch such as corn starch is used the end-product may be a gum drop, gum slice, or jelly bean. It is possible to boil out a relatively large amount of water from a formulation which contains starch, corn sirup, and sugar, leaving the starch jelly in a stiff and gummy condition. When this gum is cast and allowed to dry, the chewy type of confection results. By the use of a shorter cooking time (or by cooking to a lower final temperature) more water is left in the starch jelly, and softer confections with a "shorter" texture are obtained. The gelling power of starch is preserved and after being poured into molds to set to shape, the gum may have a moisture content of about 20%. These are the familiar gum drops or gum slices.

Gum drops are commonly made by the use of thin boiling corn starch for three principal reasons. Corn starch has excellent gelling properties and therefore is preferred to the softer setting starches such as tapioca. Sago starch has good gelling properties and has been used for this purpose. However, gums made from sago starch have a tendency to become more opaque and dull after aging than gums from corn starches which are prepared for this use. As a starch is modified by acid treatment to produce the usual thin boiling variety, the hot paste viscosity of the starch is reduced, but modifications, *e.g.* fluidity grades of 40 and 60, produce pastes which show a more pronounced tendency to gel (when considered in relation to their hot paste viscosity) than an unmodified starch. Therefore, by the use of a slightly greater amount of thin boiling starch, a hot paste viscosity results which is equivalent to that of the unmodified starch, but when the two hot pastes of equivalent viscosity are cooled, the gel strength of the thin boiling starch paste is considerably greater than the gel strength of the paste of the unmodified starch. Thus, by the use of a thin boiling corn starch it is possible to adjust the formulation so as to prepare a cooked mixture that possesses a high degree of fluidity when hot and a satisfactory gel structure when it is cast and allowed to set, even though the gel contains 20% or more moisture. A fluid hot paste is desirable, since it facilitates the pouring of the cooked starch jelly into molds. The manner in which the starch is modified to reduce its hot paste viscosity is chosen so that a "stringy" characteristic does not develop in the hot starch jelly, for if it does the flow of the gum into the molds will not break abruptly after the mold is filled and the kettle is moved to the next mold. This results in castings which are connected by thick threads of the gum, which represent a loss in the yield of finished goods.

The relative increase in gelling power which results when corn starch is acid-modified is a function of several variables employed in this conversion. Normally these modifications are carried out by converting the starch in a suspension with 0.1 *N* H₂SO₄ at 125° F. for from 10 to 15 hrs. As the concentration of the acid employed is reduced, that is as the pH is decreased, and as the time of the conversion is increased, thin boiling starches are produced (*e.g.*, starches with a fluidity of 60) which cook to measureably softer setting pastes. Conversely, as the acidity is increased and as the conversion time is reduced, a thin boiling starch is produced with more pronounced gel strength. A relatively high concentration of acid may be used to make corn starches of 40 or 60 fluidity by the use of converting temperatures lower than 125° F. An illustration in which the above effect is employed to advantage in the manufacture of a gum drop starch is the process patented by Meisel (34).

Clarity of the gum is a prime requisite for some types of confections. Although both corn and sago starches form pastes of high gel strength, both give gels which cloud in time after they are cast. Relatively clear gums can be made from corn starch after it is acid-converted to a fluidity higher than 60. However, the gel strength, per gram of the cooked starch, is also reduced and soft gums result. Oxidation of corn starch, *e.g.* by a treatment with sodium or calcium hypochlorite, or by sodium peroxide, modifies the starch so that it cooks to a very clear paste, but the gel strength is very low. On the other hand, Kerr (35) has observed that corn starch may be oxidized with calcium peroxide under special conditions to produce a starch of high potential gel strength and one which makes an exceptionally clear gum drop.

The gums are shaped after the cooking procedure by pouring the hot jelly into molds. These are made by impressing the desired shapes in a layer of starch. A special corn starch is suited for this purpose. It is usually dried to a low moisture content to increase its water-absorbing capacity, and a small amount of an edible oil is thoroughly blended with the starch so that it will retain the shape of the impressions made in it. The trays of starch into which the gums are poured are placed in a hot room to adjust the gums to the correct moisture content and to set the gel. The molded gums are then shaken loose from the starch and are freed from any remaining starch by brushing. The starch is reconditioned by screening and by an occasional redrying treatment. Fresh molding starch is added to compensate for losses.

After the gums are brushed they may be finished by rolling in sugar crystals to apply a sweet crystalline surface to the gums, or for a similar result they may be wet with a saturated sugar solution which subsequently crystallizes on the surface of the gums. The more extensively cooked gums, such as jelly beans, may be finished by a panning operation. The confections are placed in a rotating kettle where by a tumbling action a sugar coating is built up by adding a sugar sirup progressively in small amounts. After the coating has been built up to the desired extent the operation is continued so that the exteriors develop a

gloss. Frequently ingredients are added in the panning operation which act as polishing agents.

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CHAPTER XXI

USE OF STARCH PRODUCTS IN THE TEXTILE INDUSTRY

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1. Introduction. The principal uses of starch and starch products in the textile industry are for sizing yarns (warp sizing) preparatory to the weaving operations, for sizing or "finishing" the cloth after the yarn is woven, and for printing certain types of fabrics with printing pastes which are frequently a

mixture of dye and starch paste. The function of the starch product in warp sizing is primarily to protect the yarn so that it may be woven with less damage. After weaving, the size is generally removed. The woven goods are finished by a variety of processes either to make their appearances attractive or to prepare them for a particular commercial use. The function of starch products in finishing will vary with the purpose of the finishing operation. Color patterns are reproduced on some types of fabrics, as in calico, by a printing process. The function of a starch product in this operation is to thicken the solution of the dye and to act as a carrier for the color. Although each of these major applications of starch to textile manufacture is outlined, the following discussion is concerned primarily with warp sizing. This emphasis is intended to reflect the proportionate advancement which has been made in a study of the subject of warp sizing in recent years by the combined efforts of the carbohydrate chemists and textile technologists.

Warp sizing may be defined as the application of a protective coating or dressing to warp yarns prior to weaving. The purpose of the process is twofold: (a) to increase the weaving efficiency of the warp by reducing the number of warp breaks and the amount of shedding in the loom, especially with cotton and other spun yarns; (b) to improve the quality of the fabric produced by reducing the number of defects, such as knots arising from warp breaks, and, in smooth fabrics, produced from continuous filament bright rayon yarn, by preventing delustering of the yarn by abrasion on the loom parts. The process of warp sizing is also known as "warp slashing" or simply as "slashing," terms which are not descriptive and whose derivation is not clear. The general process is the same although the actual details of the procedure may vary widely according to the system used. For ease in description, the silk system, widely used on continuous filament rayon, is given below in some detail and the variations of the other systems noted briefly.

Warp sizing of continuous filament rayon is carried out on a rayon slasher, a machine which consists essentially of (a) a steam-jacketed size box or trough containing an immersion roll and a set of quetsch rolls. The latter, usually three in number, are power-driven and are arranged vertically one above the other. The lowest roll is partially immersed in the size liquor. The function of these rolls is to pull the warp from the warp beam in back of the slasher into the size box, and to squeeze the excess size liquor from the warp before the latter goes to the drying cylinders; (b) a set of steam-heated, power-driven drying cylinders, known as "dry cans," usually three, five, or seven in number; (c) a wrap-up mechanism consisting of a beam driven at the appropriate speed so the dried warp is wound up under the proper degree of tension. A press roll riding on the warp as it is wound on the beam helps to insure uniformity during this operation.

A warp consists of a definite number of warp yarns or "ends," usually about 2000 to 8000, depending on the construction of the ultimate fabric. These are arranged in parallel and are wound on a warp beam. In the slashing operation

this warp is drawn from a single beam in back of the slasher over a drag-roll into the size box by the nip of the quetsch rolls. In the size box, the yarn picks up the size liquor either by passing through it under an immersion roll or merely by passing through the nip of the two lower quetsch rolls and being wet by the size carried up to the nip by the lowest roll as it revolves partially immersed in the size. There are several ways in which a warp may be threaded through the quetsch rolls, depending on such factors as the type of yarn, the number of ends in the warp, the degree to which the yarn is to be stretched during slashing, etc.

A detailed description of the operation of the quetsch (and of rayon warp slashing in general) is given by Mauersberger and Schwarz (1).

After leaving the size box and quetsch rolls, the wet warp is dried first on one side then on the other by passing over and under the alternate drying cylinders. Steam pressure is maintained in these cylinders such that their surface temperatures are in the range of 100–212° F. The actual temperature is determined by the drying requirements of the warp; *i.e.*, the number of ends, and the degree to which the excess size liquor is squeezed out by the quetsch rolls, the slashing speed, and the preference of the operator. On a multiple can slasher it is customary to operate the machine so that the maximum temperature is attained on the middle cans, while the first and last cans are cooler. This prevents an explosively rapid drying of the yarn on the can nearest the size box and allows the warp to leave the last can at a lower temperature.

There is little control of the actual "regain" (*i.e.*, of moisture content) to which the yarn is dried other than manual control of the speed at which the warp is sized and passes over the drying cans. This depends on the experience and skill of the operator. The latter, in order to avoid underdrying of the yarn and consequent danger of mildew development, usually dries the warp to a moisture content lower than normal for the yarn. The size must therefore be of such nature that it is not decomposed or rendered insoluble by such overdrying.

The warp, after leaving the drying cylinders, is wound up directly on a loom beam at the front end of the slasher. This beam is driven at such a speed that the warp is wound up under sufficient tension to produce a firm uniform beam with no loose or slack ends. Too much tension at this point is undesirable, as it is one cause of excessive elongation or stretch of the yarn during drying. During the slashing operation, it is usually considered desirable to keep the total elongation of the yarn at a minimum, certainly not over 5%.

After the warp leaves the final drying can and before it is wound on the loom beam, the individual warp ends, which have a tendency to be glued together by the size, are separated from one another for an instant by a series of iron bars known as split-rods or lease-rods. After this operation the sized and dry warp yarns should have no tendency to stick together again, no matter how tightly they are packed on the loom beam. A size which has little adhesion to the yarn will flake off, or shed, at the lease-rods. On the other hand, a size which binds the yarns too firmly together will cause trouble here in the form of warp end

breakage. The yarns should separate readily and smoothly with a minimum amount of shedding at the lease-rods.

The lease-rods also aid in the insertion of lease strings in warps sized on the silk system. These strings keep the yarns in the proper relative order so they do not become crossed or snarled.

The beam of sized yarn is removed from the front of the slasher and placed in the loom. The ends are "drawn in" the loom, or else "tied in" at the end of a warp of the same number of ends which is just running out. The warp is then ready for weaving.

Slashers are operated at speeds varying from 20 to 60 yards per minute. The trend in modern slasher design has been consistently toward higher speeds.

In the silk system of slashing the size liquor is usually prepared in copper size kettles in batches of 40 to 100 gals. or larger, depending on the equipment available. For heating purposes the kettles may be steam-jacketed or may contain open or closed steam coils. The method of stirring may be manual or by motor-driven paddles. The following procedure is common. Water at ordinary temperatures is admitted to the size kettle up to about one-half or two-thirds of the final volume of the batch. The weighed amount of dry starch is added slowly with stirring until a uniform suspension or milk is formed. The temperature is raised by steam to approximately 212° F. and held there until the starch is gelatinized. Untreated or thick boiling starch used in cotton sizing may require heating for 15 to 30 min. or longer at the boiling temperature. Dextrins and thin boiling starch require much less heating time. When gelatinization is complete, the paste is allowed to cool to 160–180° F. and the other ingredients, such as softeners, plasticizers, penetrants, etc., are added. The volume is brought up to the value desired by the addition of cold water. The size is then ready for use.

The above procedure assures that each size mix will have approximately the same concentration of dry substance and prevents an overdilution by condensation from the steam line, if open coils are used, or loss of water by evaporation when closed steam coils are used for heating.

Heating by injection of steam from open coils also has the effect of very vigorous mechanical stirring and may decrease the viscosity.

The prepared size may be stored in the size kettle, in storage tanks, or it may be fed directly to the slasher.

The kettles are frequently located on a raised platform so the size flows by gravity from the kettle to the size box. As the size is used up by the warp, more is admitted through a valve which is either controlled manually by the operator or, in the newest slashers, by an automatic size level control.

Where several slashers are using the same size liquor, it may be kept circulating through a main line from which the slashers draw it according to their requirements. The size storage tanks and pipe lines are usually not heated or insulated, so the size may cool almost to room temperature before it reaches the size box.

The temperature at which the size liquor is maintained in the size box varies with the type of yarn sized. For rayon it is usually 140–155° F. Acetates are run at lower temperatures. For cotton yarns, the temperatures are considerably higher, 190–210° F.

When spun rayon or cotton warps are sized on the cotton system there are certain differences in the procedure, although the principle is the same. The warp is drawn, not from a single beam, but from a number of beams placed in a creel in back of the slasher. The ends from the individual beams are combined into a single sheet of parallel yarns before passing into the size box. The size box of the cotton slasher is usually larger (40 gals. as compared to 10 gals. capacity) than that of the rayon slasher. It may contain two sets of squeeze rolls rather than a single set of quetsch rolls. The drying cans on a cotton slasher are usually fewer in number but considerably larger than those on a rayon slasher. There are also certain differences in which the leases are inserted at the beginning and end of the warps.

The equipment and procedures used in cotton warp slashing are described in considerable detail by Merrill, Macormac, and Mauersberger (2).

The principle of sizing woolen and worsted yarns is the same as that in spun rayon and cotton sizing, the chief purpose of sizing being to lay the surface fiber and thus to convert a fuzzy, hairy yarn into a smooth one which will not chafe or shed fiber in the loom. The equipment is analogous to that used in the cotton system, although a drying chamber usually performs the function of the drying cylinders in cotton and rayon sizing. Only a very limited number of rayon slashers use air drying in place of cylinders. Worsted and woolen warp slashing is discussed in detail by von Bergen and Mauersberger (3).

2. Requirements of a Warp Size. The properties which a satisfactory warp size must have may be classified under two general headings, (A) paste characteristics and (B) film characteristics. The former are those properties which govern the behavior of the size during preparation and application; the latter affect its behavior during weaving and subsequently in desizing.

A. Paste Characteristics—Among paste characteristics may be listed the following requisites: (a) ease of preparation, (b) uniform viscosity, (c) absence of pronounced congealing and skinning properties, (d) pH near neutral, (e) absence of foaming properties, (f) absence of prolonged tackiness during drying, (g) compatibility with other components of the size mixture, and (h) stability towards decomposition.

(a) Size mixes are readily prepared from starches, as the latter require no preliminary soaking or swelling treatment such as may be needed with gelatin or glue sizes. If the starch is added at a reasonable rate with moderate stirring to water at room temperature, a uniform dispersion is formed. Since most starches wet out readily, there is little difficulty due to the formation of aggregates or clumps. Dextrins and very thin boiling starches form pastes very easily if the suspension is heated to 190–212° F. for 5 to 10 min. Thick boiling starches and gums require longer periods of heating at a temperature as close to the

boiling point as possible. Although the viscosity of starch pastes may be reduced by prolonged heating, there appear to be no other effects which are undesirable.

(b) After the size paste has been prepared, the viscosity of the mix should not be readily diminished by further heating or by mechanical agitation, such as may be encountered in size circulation systems and even in the size box itself. This is important, as it has been shown that changes in viscosity affect the amount of size picked up by the yarn (4). In starches of high fluidity and dextrins most of the vesicle structure of the starch grain is destroyed during the pasting operation. Consequently the viscosity of size mixes prepared from these materials is usually low, and further changes do not take place even with severe mechanical agitation. A comparison by Katz of the relative viscosities of pastes of various commercial starches has demonstrated that corn, wheat, and sago starches produce pastes which are much less affected by heating and stirring than those made from potato and tapioca (5). The effect of these factors on the viscosities of thin boiling corn and sago starch pastes is also discussed by the same author (6).

(c) The size may cool considerably in the storage tank or in the pipe lines to the size box. This should not cause the size to form a permanent gel which cannot be redispersed readily on warming, otherwise lumps of gel may be picked up by the warp in the size box. These gel particles dry to hard spots which may cause a number of yarns to break out at once at the lease-roads, or may result in irregularities in the woven fabric. Thin boiling starches have less tendency to gel than native starches, and the tendency decreases with the extent to which the starch has been modified. Dextrins and very thin boiling starches form pastes of low viscosity which have practically no gelation tendencies at the concentrations at which they are used.

A phenomenon which may be related to gel formation is the formation of a skin or film of congealed paste on the surface of the size mix. It occurs when considerable evaporation may take place from the exposed surface of the size paste, for example in the corners of the size box, or in uncovered storage tanks where there is no stirring. These pieces of congealed size cause "hard size" spots on the warp and lead to the difficulties noted above. Dextrins do not exhibit this undesirable property to any appreciable extent. However, pastes of native starches, thin boiling starches, and gums are apt to form skins to a greater or less extent.

(d) The pH of the size mix should be preferably in the range near 6 to 7. Sizes which are alkaline are apt to promote foam formation, as the flannel blankets on the quetsch rolls produce small bubbles which quickly build up to large amounts of foam especially at high slashing speeds. This foam is troublesome as it may fill the size box and conceal the true liquid level. The latter, whether controlled either manually or automatically, may drop so far that there is not enough size liquor provided in the box. Then the yarn does not pick up sufficient size and the result is a "soft warp" with poor weaving qualities.

Excessively high acidities are harmful to the yarn, as they are apt to lower the tensile strength, especially of viscose and acetate rayons. Pastes made from

starches and dextrins frequently are as high in acidity as pH 3 to 4. Materials intended for warp sizing are therefore often buffered to adjust the paste to about pH 6, by the addition of very small amounts of borax or sodium carbonate to the dry starch before it is sold. These added materials usually also promote the film-forming properties of the size.

(e) The size should not contain any material which would promote foam formation. Starches and starch products, unless buffered to above pH 7 show little tendency to foam. The tendency is further inhibited by the presence in the size mix of the oils and fats (usually sulfonated) which are added as softeners and plasticizers for the size film.

(f) As the size film dries on the yarn it should pass very quickly through the tacky stage, otherwise it is apt to stick the yarn to the drying cylinders causing the "ends" to break and wrap around the cylinder. Dextrins which contain a high percentage of cold water-soluble material may exhibit this phenomenon more than other starch products. However, they are not used to a large extent in warp sizing as their film characteristics are not usually satisfactory.

(g) The water with which the size paste is prepared may be of varying degrees of hardness, depending on the district in which the mill is located. Usually no effort is made to soften this water prior to slashing. The size should not therefore contain any materials which will react with the salts in the water to form insoluble materials which may be precipitated on the yarn. These would not be readily removed in desizing processes and would result in uneven dyeing. Starches give little trouble in this respect.

The size should also be compatible with materials, such as oils, fats, and their sulfonated derivatives, waxes, glycerols, and other humectants, pine oils, and similar penetrants which may be added to the size mix for specific purposes. Some of these may cause an increase in viscosity of pastes made from alkali gums, but otherwise little difficulty is encountered.

(h) Since the size liquor is prepared in batches, it is often used up within a few hours but it is not usual for the size to be stored for long periods of time. Therefore, a size mix should show as little tendency as possible to undergo decomposition under slasher room storage conditions. Sizes containing glue and gelatin materials may be less stable toward decomposition than starch sizes, and consequently preservatives such as cresylic acid or its derivatives are often incorporated in their mixes.

B. Film Characteristics—The size must be essentially a film-forming material and the film should possess to some extent the following properties: (a) tensile strength, (b) adhesion to the yarn, (c) flexibility and folding endurance, (d) hardness, especially resistance to abrasion, (e) insensitivity to overdrying, (f) moderate insensitivity to changes in relative humidity, (g) little tendency to develop static electrical charges on abrasion, and (h) ready resolubility regardless of the age of the size film, for ease in desizing.

(a) Although the tensile strength and the elongation of films of sizing materials have been studied in some detail by Neal (7), Farrow (8), Furry (9), and

others, no well defined correlation has been found between these properties and the weaving properties of a sized warp. It is highly probable that no simple relationship exists between any single film property and the behavior of the film on the yarn in the loom. When the results of work now in progress (10) are available for publication, it is indeed likely that the relationship between film properties and weaving efficiency will be considerably more clearly defined. In general, materials which form films with sufficient tensile strength to be self-sustaining may be considered as possible warp sizes if their other film and paste properties are satisfactory. One exception to this conclusion is a reported use of a linseed oil derivative which has been employed in Europe especially for sizing continuous filament rayon. However, it has not been used to any appreciable extent in the United States.

(b) Whether the size is applied as a rather heavy coating on the outside of the yarn, as for example on cotton, spun rayon, and other hairy yarns, or whether the size is present either in the form of a thin film lying mainly between the filaments or in the form of a matrix in which the filaments are embedded, as in continuous filament rayon sizing, the size film must adhere tenaciously to the filament and fiber surfaces. If the size film lacks this property, it will flake off or "shed" from the yarn at the split-rods at the front of the slasher, and in the loom at the dropwires, heddles, and reed, where the yarn is abraded and flexed by a contact with the loom parts. This loss of size exposes the yarn to the very abrasion from which the size is intended to protect it and a low weaving efficiency results. Starch films adhere well to the fiber surfaces of wool, cotton, and viscose rayon, both the bright and delustered varieties. However, as yet no starch product has been developed which by itself has the requisite adhesion to the filament surfaces of acetate rayon, nylon, and vinyon yarns to provide satisfactory sizing. The development of such a starch product or other materials which will function to anchor the starch film to the surface of the filament is as yet an open field for study.

(c) During the weaving operation the yarn is flexed rapidly and repeatedly at sharp angles. Consequently, the size film must be flexible enough and have sufficient folding endurance to enable it to bend with the yarn. A study by Furry (9) of films of materials which are used in sizing and finishing has shown that a series of seven starches can be listed in the order of a decreasing endurance to folding as follows: canna, sweet potato, potato, corn, rice, wheat, and dasheen. Film flexibility is usually increased by the addition of so called plasticizers and softeners. These are materials such as oils, fats, waxes, and, when greater ease of removal from the yarn is essential, the sulfonated derivatives of these materials. The amount of softener used depends entirely on local conditions of sizing and weaving which vary from mill to mill. These conditions involve such diverse factors as the type of yarn sized, the construction of the fabric to be woven (*i.e.*, the number of warp and filling yarns per inch), and the type and age of the looms in the weave room. In general, for starch sizes it is desirable to maintain the content of softener at an amount not exceeding 5% of the starch

content of the size, otherwise other film properties, such as resistance to abrasion, will be impaired.

(d) Little information has been available on the relation of film hardness and resistance to abrasion to the weaving efficiency of the sized yarn. It has been generally known that an excess of softener in the size will produce a size film which is soft and mushy in character and which will not protect the yarn against abrasion in the loom. The operator is always confronted with the dilemma of employing enough softener to produce a flexible sized yarn, without, however, adding so much softener that the yarn becomes soft and fuzzes badly in the loom. In the past, the solution of this problem has been by trial and error. The research now in progress under the auspices of the Textile Research Institute (10) is expected to develop valuable information on the relation of film hardness of sizing materials to the behavior of the sized warps during weaving.

(e) It has been pointed out above in the discussion of slashing that, in general, warps tend to be overdried as they leave the slasher. It is important, therefore, that the size be of such a nature that it is not readily affected by such abuse. Starch materials may tend to undergo a slight degree of conversion to gums and dextrans when they are overdried. This effect is not serious, as the materials produced in this manner are usually even more readily soluble than the original starch products. Sizes containing protein may, however, be rendered less soluble by such treatment.

(f) The air in the weave rooms is humidified in order to maintain desirable yarn properties, particularly those which promote good weaving. In weave rooms where viscose rayon is woven the relative humidity is generally maintained in the lower portion of the range 55 to 65%; for acetate rayon and cotton the relative humidity is considerably higher. However, a close control of the relative humidity is usually not possible over the extended period of time necessary to weave a single warp and the humidity may vary considerably, especially when the weave room is shut down, as it usually is under normal conditions over week-ends. The size film should be of such a material that its properties are not too grossly affected by a wide variation in the relative humidity; that is, it should not become tacky at a high humidity, nor brittle at a low humidity. Starches are analogous to other carbohydrate materials, such as cellulose, in their behavior under various conditions of humidity. They are softer and more flexible at high than at low relative humidity. Consequently the behavior of the yarn, in so far as atmospheric moisture is concerned, is not changed greatly by sizing with starch products. Only with a few types of yarns is it considered necessary to increase the humectant properties of starch sizes by the addition of hygroscopic materials such as glycerol, sorbitol, etc. Sizes for cotton are most apt to contain such materials.

(g) Since the process of weaving involves considerable friction, yarn against yarn and yarn against metal, there is an opportunity for the development of static electricity. This effect is not generally a problem, except with acetate rayon. In weaving acetate yarn, the filaments may become so highly charged

as to repel each other. This may cause defects in the woven fabrics when a highly regular, smooth surface is desired. The size material should be of such a nature that it inhibits rather than contributes to the development of a static charge during weaving. Starch sizes appear to be satisfactory in that they do not cause static during the weaving of viscose rayon and cotton. As they do not have a high degree of adhesion to acetate yarn they are not usually employed for sizing acetate rayon. In cases in which they have been tried as sizes for acetate, the starches did not appear to affect the development of the static charge in any way. Gelatin sizes also do not appear to inhibit the formation of static electricity during the weaving of acetate yarn. The addition of humectants to the size and the use of high humidity during weaving tend to reduce the tendency of acetate warps to develop static charges during weaving.

(h) Very few fabrics are sold in the state in which they come from the loom. They are usually desized, dyed, and finished in subsequent operations. A few exceptions are denims, plaid, and figured materials, which are woven from yarns previously dyed. The warp size must be removed prior to dyeing, or else uneven dyeing and dye-resistant marks usually occur. The ease with which the size film is resolubilized is therefore an important size property, especially in respect to the processing of rayon fabrics. Cotton goods can be given a vigorous desizing treatment, but rayons, because of their low tensile strength when wet must be handled more carefully and cannot be subjected to the action of strong alkalis or acids. It would be desirable if the size were so readily solubilized that it could be removed from the fabric merely by rinsing the goods in warm water containing a small amount of soap to remove traces of oil and grease which the fabric may have picked up accidentally. Although some dextrans are desized by very mild soap and hot water (a "boil-off"), many finishers prefer to use an enzyme treatment to insure a complete removal of the starch in a shorter time and with less possibility of damage to the fabric. The use of enzymes for solubilizing starches in textile operations is too extensive in subject matter to be considered in detail here.¹ The warp is usually immersed in a solution of the enzyme (about 5% concentration) and allowed to remain in contact with this solution at the optimum temperature for the activity of the enzyme until tests on small swatches of the cloth no longer show the typical blue coloration given by starch and iodine. The enzyme and the solubilized size materials are rinsed from the fabric which is then submitted to the dyeing and finishing operations. The temperature at which each enzyme functions best is a characteristic of the enzyme and specific directions for its use are best obtained from the manufacturer. Many are active in the temperature range of 50–60° C. Too high a temperature inactivates the enzyme; too low a temperature greatly extends the time required for desizing. The time required for the complete removal of the starch product varies with the amount and type of starch on the yarn and on the particular enzyme used. A period of 30 min. to 1 hr. is usually sufficient.

¹ See Chapters XV and XVI.

The enzymes used in desizing may be simply diastatic in action, such as Diastafor, or they may also exert a proteolytic action, as for example, Degomma. Their sources are diversified, including various animal glands, malt, bacteria, and molds. Among the commercial enzymes commonly used are the following: Degomma, Diastafor, Exsize, Polidase, Polyzime, Rapidase, Serizyme, and Wiazyme. Also, Arcy is extensively used in the textile industry but more frequently it is used to convert native starch in preparation of the size.

Other materials which are not enzymes but which react with starch to solubilize size films are often used. An example of such a reagent is Aktivin, the sodium salt of toluene chlorosulfonamide. This compound oxidizes the starch to soluble and readily removable substances. It also exerts a bleaching effect on the yarn.

3. Sizing of Continuous Filament Yarn. The problems arising in the sizing and weaving of continuous filament yarns, such as viscose and acetate rayon, vinyon, etc., differ markedly from the problems presented in sizing spun yarns such as wool, cotton, and spun rayon and acetate. In the first group the yarns are smooth, the filaments continuous, and the yarns have just enough twist (usually 3 to 5 turns per inch) to hold the filaments together. In sizing these yarns it is not necessary to make the yarns smoother, but only to bind the filaments lightly together in order to prevent a broken filament from being pushed back along the yarn to form a "fuzz-ball" or "bird's nest" as it is frequently called. Also, another purpose of sizing is to protect the outside filaments from delustering by the abrasion on the loom parts.

Gelatin and gelatin glues were formerly used almost entirely on continuous filament rayon. More recently, however, it has been found that white dextrans, both corn and tapioca, can be used satisfactorily as sizes for viscose rayon yarn. Dextrans from potato and sweet potato have been experimented with sporadically but they are not extensively used at present.

Although films of dextrinized starches are not as strong and flexible as films of the original starches from which they are derived, the dextrans, unless they are too highly converted, have sufficient film strength to bind the filaments together satisfactorily for good weaving. The dextrans have the advantage in that they are much more readily desized than the native starches. It is not advisable to try to give specific sizing formulations, as the conditions for sizing and weaving vary so much from mill to mill that a formula found to work successfully in one place often cannot be used in another without extensive modification. The size is usually applied from a mixture which possesses a low viscosity and contains from 35 to 50 lbs. of dextrin per 100 gals. of finished size. The amount of size which is picked up by the yarn may vary considerably, but the usual range is about 4 to 7% of dry size on the yarn and 5% is a common value. Generally a softening agent is employed with the dextrin, such as a readily dispersible sulfonated oil or tallow. The softener is used in amounts less than 5% of the weight of the dextrin. The size box is maintained at a temperature in the range of 140-165° F. The preparation of the size follows the general procedure given

previously. A heating period at 190° F. for 15 min. is sufficient to disperse the dextrin completely. Mixtures of dextrans and gelatin, or gelatin glue products have been used satisfactorily in the sizing of viscose rayon. In general, highly converted dextrans, such as the canary yellow type, have not proved successful for viscose rayon. These materials appear to pass through a prolonged tacky stage when they are cooked and dried and consequently may give trouble by causing the yarn to stick to the drying cylinders on the slasher. These highly colored dextrans may also impart undesirable tints to the yarn which are not removed in the desizing process. By themselves, no starch products, including dextrans, have shown sufficient adhesion to the yarn surfaces to provide for a satisfactory weaving of acetate rayon, nylon, and vinyon. However, dextrans are frequently incorporated in gelatin and gelatin glue sizes for sizing acetate yarns.

4. Sizing of Spun Yarn. The successful application of a size to spun yarn requires that a hairy yarn be converted into a smooth one, with as few projecting filaments and as little loss in flexibility as possible. This requires size mixtures of comparatively high viscosity. Size mixtures such as those which work satisfactorily with continuous filament yarns merely saturate the porous spun yarns and on drying leave them stiffer than before sizing but still as rough and fuzzy. Many reports have been written on the problem to determine whether the size should penetrate into the interstices between the filaments or simply coat the surface of the entire yarn. However, satisfactory weaving has been achieved with warps on which the size is largely on the yarn surface and has only penetrated far enough to anchor this surface film securely to the yarn. Too much penetration of the size into the interior of the yarn appears to stiffen the yarn unduly.

Native, or unmodified corn starch was formerly used to a large extent to size cotton yarn. Thin boiling or modified starches were developed more recently in order to provide products which have a lower viscosity for the same amount of dry substance in the size and which have less tendency to form gels in the size box and pipe lines supplying the size when the temperature of the size is lowered. In conjunction with a small amount of softener, thin boiling corn starches are perhaps the most widely used sizing material for cotton warps. The concentration of size varies considerably, depending on local conditions. About 0.5 to 1.0 lb. of dry size per finished gallon is a range of concentration frequently encountered in practice. The amount of dry size added to the yarn may be in the neighborhood of 8 to 10%. The softener is employed in varying amounts up to 5% of the weight of the starch. The former is usually a tallow or wax or occasionally a sulfonated derivative of the fat. Thin boiling starches which have been manufactured by an acid modification and those made by an enzymic conversion are the types most commonly used. Oxidized starches have not been extensively used for the sizing of cotton warps.

In so far as sizing and weaving alone are concerned, acid-modified, thin boiling starches would, no doubt, be satisfactory for sizing viscose rayon spun

yarns. However, the ease of removal of sizes made from these thin boiling starches is not sufficiently great to permit their general use. They require a desizing treatment which might be injurious to rayon. Instead, starch gums, the so called British gums, are used for this purpose to a large extent. These gums form pastes which exhibit high viscosities, comparable in this respect to the native starches, or to thin boiling starches of low fluidity. However, the films formed from British gums are considerably more readily dispersed, and, consequently, warps which are sized with them can be readily desized by a mild enzyme treatment. A size concentration of 0.5 to 0.6 lb. per gallon is commonly used in practice. This concentration is normally sufficient under ordinary sizing conditions to allow 6 to 8% of size to be taken up by the yarn. Highly dispersible, sulfonated oils are used as softeners. Frequently additional materials, such as pine oil, are added in small amounts as penetrating agents, especially when the spun yarn is a blend of viscose and acetate fibers. For these fiber blends many size mixtures have incorporated in them considerable quantities of gelatin or gelatin glue; the more the acetate in the blend, the higher is the percentage of gelatin used in the size mixture. For sizing spun yarns, especially cotton as noted above, some mills prefer to make their own thin boiling starches from native starch by treating the starch paste with an enzyme prior to sizing. Other starch-modifying agents, such as Aktivin, are preferred by some mills. Conversion of starch at the textile mill is a procedure which requires considerable skill and careful control in order to achieve uniform results. Properly executed, however, this procedure appears to produce satisfactory sizes.

Thin boiling corn starches of low fluidity find considerable use in the sizing of warps of single ply worsted or woolen yarn. Plied, or highly twisted woolen yarn, is frequently woven without size.

The rapid development of the synthetic fiber industry in the past few years has stimulated considerable research and investigation of the methods and materials used in sizing, especially rayon sizing. Many mechanical improvements have been developed which have increased the sizing speeds and which have led to a closer control of the process mechanically. Materials for sizing have been improved so as to provide sizes which are applicable not only to single types of fibers, but to a variety of blends of natural and synthetic fibers.

6. Finishing Textiles with Starch Size. The principal function of starch in finishing textile fabrics is to impart or accentuate the desired physical characteristics in the cloth. When the large variety of fabrics which are manufactured is considered, it may be appreciated that many modifications of starch are required to obtain the various results desired.

Starch was originally used primarily as a stiffening agent for fabrics; that is, its function in this case is to increase the structural strength of the yarn and to lock the yarns in a fixed position with relation to each other in the fabric. When no other characteristic is desired except possibly to add weight to the fabric, an unmodified starch, such as corn starch, is suitable for the purpose. Thus,

unmodified starch is suitable for sizing certain types of gauze, netting, cloth used to cover hat frames, and some of the other loosely woven fabrics.

If in addition to "yarn fixation" the added characteristic of flexibility is required, the starch is modified by procedures which will give the combined results desired. For example, a flexible finish is required for stiffening dress goods such as organdies so that apparel made from the fabric will not readily crease when it is worn. The added property of flexibility is usually obtained by procedures which reduce the gel-forming properties and, in general, the viscosity or body of the starch paste. As a result of this modification, however, more starch (weight of starch to fabric) is required to produce an equivalent amount of stiffening action. Frequently added weight is an advantage, since fabrics may be sold on the basis of weight rather than yardage.

One of the more recent developments in the art of finishing textile fabrics, known as sanforizing, involves an unusual combination of the effects described. In this operation the goods are shrunk by the application of moisture and hot irons and the yarns are "fixed" by the application of a starch size. However, a flexible rather than a "board-like" finish is usually required for most of the fabrics which are sanforized. Highly converted corn starches (acid-modified to a fluidity of 90), chlorinated corn starch, and dextrans are commonly used to perform the two principal functions of the size in this finishing operation. In addition to the other properties of starch which are required (depending on the type of fabric sanforized) the starch product should not produce a size which, after application, will stick to and tend to caramelize on the hot sanforizing irons.

In general, a flexible finish is more often desired for fabrics than a harsh, "board-like" finish. Even in instances in which a high degree of stiffening is required, flexibility may be essential so that when the fabric is folded, as in the manufacture of shirts and collars, the yarns will not be broken or injured. Therefore, starch products which set to soft gels when gelatinized are normally preferred. It is a natural consequence that the non-cereal starches, particularly potato, should have been used to a large extent in finishing. One of the principal disadvantages of the use of potato starch is that its paste viscosity is not stable; that is, the viscosity tends to decrease when the paste is boiled or held at elevated temperatures or stirred vigorously. This instability is magnified in alkaline media and it is a common practice to add borax to finishing sizes in order to increase the brilliancy of the finish. Therefore, since the results obtained in finishing are to a large extent related to the viscosity of the size, the use of potato starch leads to variable effects: the first part of a batch of the size gives results which are different from those obtained by the use of the later part of the batch. In order to maintain a greater degree of stability and also to produce a firmness of finish which cannot be obtained with unmodified potato starch alone, more recent practice involves the use of a potato starch which is modified (*e.g.*, with acid to a fluidity of 20) purposely to reduce its paste viscosity. Less apparent instability of paste viscosity results and the latter is accordingly, but erroneously, said to be "stabilized." In addition, owing principally to the greater weight of

size which may now be added, some improvement in the firmness of the finish is obtained. Another preferred procedure is to add corn starch or sago flour to the potato starch in order to increase the paste stability and firmness imparted by these starches.

Wheat starch has been used extensively in finishing. Although sizes made from wheat starch do not possess the degree of instability in paste viscosity that is shown by sizes made from potato starch, they also do not impart the brilliancy and flexibility of finish which is obtained by the use of potato starch. Rather satisfactory finishes may be made by the use of corn starch gums and chlorinated corn starch products. For example, a size that is suitable for finishing fabrics such as twills and denim is made from corn starch by a procedure described by Kerr (11) in which the starch is allowed to be converted in the presence of chlorine gas until it has attained a paste viscosity corresponding to a fluidity of 10. Satisfactory products may also be made by the use of hypochlorite to modify the starch. Representatives, presumably, of this class of oxidized starches are sold under the trade names of Beatsol, Hercules gums, and Clinco products.

The general characteristics usually desired in finished fabrics are that they possess firmness and give the impression of solidity, which is usually described as "fullness." These characteristics are not to be confused with those of a weighted or filled cloth, the production of which is outlined in a following paragraph. The characteristics under discussion are difficult to define and are often referred to in the textile industry as the "feel" or "hand" of a fabric. Pierce (12) has discussed the "hand" of cloth as a measurable quantity and Dreby (13) gives physical methods for evaluating the "hand" of fabrics.

From these discussions it is apparent that all the results accomplished in the finishing operation cannot be obtained by the use of one starch product alone. Therefore, finishing sizes are usually made of a combination of starch products. To secure special sizing effects, the size formulation may contain adjuncts such as soaps, fats, sulfonated oils, and other materials which in many cases act as plasticizers for the applied size. The study of plasticizers for starch products assumes importance in this connection. In view of the development of many varieties of such agents in recent years, the immediate future should produce significant advances in the formulation of superior textile finishes from starch products.

Frequently no more is required of a finishing size than that it add to the weight of the fabric. Usually a highly converted starch product is used for this purpose, since to make the applied size less detectable, penetration of the size into the yarn is necessary. The penetration of a size is related to its viscosity. Therefore, a reduction in the viscosity of a starch product, as for example by acid hydrolysis, permits loading of the fabric with a high percentage weight of size and permits application so that the size is neither visible nor perceptible by the "feel" of the stiffening effect imparted by unmodified starch. In the extreme cases, such as the loading of goods for rough working clothes (over-alls), very highly converted acid-modified dextrins are used and as much as a 25% increase

in the weight of the fabric may result. Sizes made from enzyme-converted starch are also used for the purpose of weighting fabrics.

In some cases the loading of goods is not primarily to increase the weight but to fill the interstices between the woven yarns. The back-filling of sheeting fabrics and of rug bases are two examples of this application of sizes. For this use an unmodified non-cereal starch such as potato or tapioca or a slightly modified starch can be used. For certain types of back-filling tapioca dextrins are used. For back-filling Wilton rug bases, for example, a tapioca dextrin made by enzymic conversion is considered to be a superior sizing agent. It is claimed that this product produces the flexibility and durability of surface which is required in a rug base.

In order to provide a complete filling of the fabric, to increase either its weight or its opacity, inorganic salts, clays, and other pigments may be incorporated in the starch size. As an example, window shade fabrics are filled with a size consisting primarily of clay, pigment, and either a corn starch dextrin, suitably plasticized, as the adhesive or a dextrin possessing a similar adhesive value and flexibility of a size film.

An additional characteristic required of a finishing size, particularly of those applied to dyed fabrics and to those made from synthetic polymers is that the size should be transparent after application and drying. Otherwise the color of the dyed fabric may be dulled or altered in shade or the luster of the goods might be impaired. Starches or starch modifications which show a pronounced tendency to retrograde² are, therefore, not as suitable in this respect as those starch products which do not. For this reason, oxidized corn starches and some types of gums and dextrins are preferred for finishing dyed goods or fabrics possessing a high sheen. No doubt in the future there will be an extension of enzyme technology to prepare suitable starch sizes for this and other special textile finishing operations.

7. Printing Pastes. Multicolored textile materials may be made by printing designs on woven goods as well as by weaving fabrics with dyed yarn. The reproduction of colored patterns is usually accomplished by the use of engraved rollers and hence the art is termed printing, although some processes involve the use of stencils and air-pressure sprays. Printing pastes are made by mixing dyes and other necessary chemicals with a water solution or paste of starch, gum, or other suitable colloid. These are called thickeners or carriers. Starch products are widely used for this purpose.

The principal function of starch in printing pastes is to act as a carrier for the dye and the chemicals required to develop and fix the dye. Inasmuch as the colors are in most cases transferred from rollers, the paste of the starch product should possess the required body, plasticity, and other physical properties so that the printing paste will operate on the rolls. It should feed smoothly into the design on the rollers and remain in place until it is transferred to the cloth. Wheat starch and wheat starch products have been extensively used in

² See Chapters VII and VIII.

the past, principally because of the excellent working qualities of their pastes. As the paste is transferred to the cloth, it should penetrate the yarns to the desired extent but it should not spread along the fibers to deform the pattern. Since the rollers apply the paste by a slight pressure, a limited amount of thixotropy is desirable, since as pressure is applied this effect favors the penetration of the color and as the applied pressure is removed, the color then tends to remain in position. Printing is applied to many fabrics which vary not only in their construction but also in the type of yarn from which they are woven; for example, fine silks, cotton, rayon, and acetate fabrics. Each of these variables affects the penetrability of the cloth, and an appropriate starch product is selected for printing, accordingly. The penetration of a paste depends largely on its viscosity; the lower its viscosity the better its penetrating power, but occasionally for some fabrics it becomes necessary to add a penetrant (*e.g.*, a glycol) even to a starch product of very low paste viscosity. The viscosity of the pastes of starch products is very materially altered by the pH of the medium and both acid and basic dyes are used in the printing of textiles. However, by far the greater portion of printing is done with vat dyes and an alkaline printing paste. Corn and some of the other cereal starch products are the most stable in an alkaline medium; the non-cereal starch products such as potato are the least stable. As a general rule, dextrins, and in particular the British gums, are more alkali-stable than their corresponding parent starch. In the printing of the better grade of fabrics, such as dress goods, it is desirable that the colored pattern completely penetrate the fabric. The color reproduction thereby simulates one woven into the fabric and in addition the colored design is less readily lost when the fabric is washed or otherwise cleaned. For less expensive fabrics, such as calico, and decorative fabrics, such as cretonne, a surface printing often suffices, indeed, is frequently preferred, since for the same amount of dye and printing paste used a better "color value" is obtained; the color is localized on the surface. In the former instance, highly modified starch products such as dextrins are used, whereas in the latter, unmodified starch or flour is satisfactory.

In many cases it is desirable to remove the starch or other colloid after the printing process. Dextrins and modified starches are naturally more readily removable by washing than unmodified starch. Dextrins are also preferable when the thickener is not removed in that their presence does not adversely affect the "feel" of the finished fabric to produce a hard, stiff finish. Starch is preferable when the full stiffening effect of the paste in the finished fabric is desirable. Also, when printing is done with pigments, the thickener is used to fix the color and in this case the ready removal of the starch product by washing is neither required nor desirable.

A disadvantage to the use of starch products as thickeners is that to obtain the proper paste consistency it is necessary to add as high as 15% of an unmodified starch or flour and a correspondingly larger amount of modified starch of lower paste viscosity to prepare the printing paste. A high content of solids in the printing paste leads to a poor "color value." Natural gums, such as tragacanth,

form relatively thick pastes at a definitely lower concentration. However, their cost is many times that of starch. Accordingly, mixtures of a starch product and a natural gum have frequently been used in order to obtain a better "color value" than can be obtained with starch alone. The more recent practice has been the use of starches of superviscosity, prepared for this purpose; that is, the use of starch products of abnormally high paste viscosity so that materially less dry substance is necessary to produce the body which is required in a printing paste. In addition, these special starches for textile printing form pastes with excellent working characteristics and possess a paste viscosity which is relatively more stable under the adverse influences of acid, alkalinity, or other added chemical. The production of starches of high paste viscosity and a discussion of the lability of these starches in the presence of acid or alkali, as the case may be, are given in a previous chapter (Chapter VI).

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CHAPTER XXII

STARCH ADHESIVES

ALEXANDER FRIEDEN

1. Introduction.

A. Definitions—At the outset it should be realized that the preparation and use of adhesives have been inexact and very much a matter of rule of thumb. If the chemist working with adhesives is to master this art and science thoroughly, he must have a broad knowledge in many allied fields. He must know something of the chemistry of paper, wood, textiles, metals, and resins, since it is the surfaces

of these materials which must be joined together. He should be familiar with the subject of molecular forces, of surface phenomena, and of interfaces. He must have a good understanding of colloidal behavior, plasticizers, wetting agents, and above all he needs to know the theoretical conceptions of adhesive substances and of adhesion.

The art of the preparation and use of adhesives is centuries old, although the science is very young, so young, in fact, that many of our present authorities on commercial adhesives fail to give recent theoretical studies sufficient consideration. Early editions of Thorpe's Dictionary of Applied Science define adhesives simply in the functional sense, as "substances or preparations of gummy or gelatinous character used for the purpose of joining together or effecting the mutual adhesion of surfaces of bodies." Brown and Truax (1) formulated a definition which takes into account theoretical considerations: "Any material which can be obtained as an emulsoid sol of suitable consistency which wets a particular surface and which subsequently forms a strong elastic jelly by cooling, heating, evaporation, or chemical reaction, must be regarded as a glue for that surface." This definition was formulated about 20 yrs. ago. It is extremely significant that few theoretical studies on adhesives date back more than 25 yrs.

This chapter is limited to adhesives made from starch, a group of adhesives that has become extremely varied both in composition and in application. The importance of this group to the industry has grown steadily, so that now there are few manufacturing processes requiring adhesion in which starch adhesives are not used to a greater or lesser extent. The multiplicity of modifications and applications of starch adhesives will be shown as we progress with our discussion, though no special effort will be made in this chapter to give formulae. The purpose will be rather to show the rational bases for the different types of adhesives, and to point to the underlying principles, so that the student of adhesives may be able to analyze his needs in the light of fundamentals and deduce the direction in which lies the probable solution of his particular problem.

The scientist is often plagued by confusion in terminology. In the case of starch adhesives, there is considerable vagueness in connection with terms to be applied when starch or starch derivatives are treated with water, with or without heat. The resultant product is definitely not a solution, unless the starch decomposition has progressed so far that the end-product is a sugar, in which case it is no longer an adhesive in the accepted sense. As starch and starch products short of sugars are colloidal in character, colloidal nomenclatures should be used. Aqueous adhesives would therefore be hydrosols or hydrophilic dispersions (2). When the hydrosols become jelly-like, through evaporation, temperature changes, chemical reactions, etc., they are hydrogels. When completely dried out, they should, provided they still retain the property of redispersion in water, be known as xerogels, a term suggested by Freundlich (3). It is also desirable to utilize the term "peptization" for all cases in which a substance is dispersed in water to a hydrosol or hydrogel, rather than use the misleading term, "solution."

B. Theoretical Consideration of Adhesives—Bancroft (4) considers that adhesion in general depends upon adsorption of the adhesive on the surfaces which are joined, and that the degree of adhesion can probably be measured by the amount of adsorption. Adsorption plays an important part in adhesion. It is, however, too inclusive, and to explain all adhesive phenomena by it is an oversimplification of the factors involved. It is now generally established that there are two types of adhesion; namely, specific and mechanical (5). Specific adhesion involves a force of attraction between the adhesive and the surface and is particularly noticeable for highly polished, smooth surfaces. Mechanical adhesion involves the imbedding or "keying" of the adhesive into the crevices of the porous material. Although mechanical adhesion is possible only with porous surfaces, specific adhesion is effective on surfaces that are non-porous as well as porous.

Bikerman (6) in discussing problems of adhesion illustrates graphically specific and mechanical adhesion, even though in an indirect way. If a crystal lattice is broken into two parts and the parts brought together in exactly the original position, the restored whole should show no break and be exactly like the original, as the known forces between the atoms and molecules are reversible. That this is not so is due to two factors: the fact that when the solid is fractured, the new surfaces, on exposure to the atmosphere, readily adsorb air molecules or react with oxygen or moisture of the air. Thus the fracture cannot be rejoined because one cushion of air is merely being pressed against the other. The adhesion of air is negligible, and the pieces fall apart. However, if this were the sole reason why a fracture could not be rejoined, a vacuum could be used to give a bonding similar to the original, and such is not the case. There is, therefore, a second cause for this failure, which is that when a solid is broken the surfaces of separation are neither flat nor smooth, and when an attempt is made to put them together again it is impossible to place every protrusion on one surface exactly in the indentation of the surface from which it was broken. The surfaces touch each other only at a few points, and the total area of contact is a very small fraction of the area of contact before the fracture. Therefore, the force required to separate the rejoined fractured body is a very small fraction of that required to produce the fracture.

The adhesive material that will overcome the molecular film of contamination on a fractured surface does so through specific adhesion, and an adhesive which will fill out all the holes and crevices of the solid, thus establishing contact along the whole surface of the fracture, operates through mechanical adhesion.

Specific adhesion is produced by molecular forces. The results of a large number of quantitative tests on joints made with pure chemical substances as the adhesives (7) indicate that the fields of molecular attraction of the opposing surfaces are superimposed upon the ordinary cohesive forces between the molecules, provided the film is very thin, although the effective range of molecular attraction may be hundreds of molecular diameters. There also appears to be a close connection between adhesion and the structure of the molecule. Thus,

in the study of pure chemical substances as adhesives between polished surfaces, the aromatic compounds have definitely more adhesion than those of an aliphatic nature (7). The molecular arrangement is also apparently a determining factor, and maximum strength may be expected with disorderly molecular arrangements (8).

Mechanical adhesion is produced by the "keying" of the adhesive in and around the fibers and the pores of the materials joined. There appears to be a considerable difference of opinion among investigators on the rôle of specific and mechanical adhesion in joints of porous surfaces. Clark (9) concludes from a study of the structure of glue films (gelatin) by x-rays that adhesion is a mechanical solidification of the gel around and upon minute fibers, because the adhering films of glue showed no property essentially different from the block of the material. On the other hand, de Bruyne (10) emphasizes the specific nature of glue joints. Brown and Truax (1) also feel that specific adhesion plays an important part in glue joints, such as those of wood. It is quite logical that in porous bodies mechanical predominates over specific adhesion. We can, however, think of few cases of adhesion that could be considered mechanical only, since the nature of adhesive forces should be considered similar to chemical affinity. Without the chemical affinity between the adhesive and the surfaces, there would be no adhesion except that due to the physical "hooking" effect of the adhesive.

It was mentioned above that Bancroft considered adsorption to be the all important factor in adhesion. This was thought to be too inclusive. Von Buzāgh (11) has analyzed "adhesion through adsorption" in greater detail, at least as it functions in the case of adhesion of microscopic particles on a plate of the same substance. His conclusions are that adhesion is the result of the interaction of surface films and the adsorption layers. Any factor that will affect the properties of the surface films will affect the adhesion. Such factors are the chemical nature of the joined surfaces, molecular structures, size of units, etc. Of particular interest is his study of the effects on adhesion of added substances which tend to modify the structure and composition of the adsorption film, such as strong electrolytes, non-electrolytes, and colloids. The results show that the basic electrical forces operating in "adhesion through adsorption" are very much the same as those in specific adhesion.

The thickness of the adhesive film is extremely important in determining the strength of the bond. Schnurmann (12) has made an interesting theoretical study of the film factors involved in adhesion, and particularly in specific adhesion. Surfaces which are optically flat may nevertheless have many irregularities. The optically flat surfaces employed by him had irregularities of the order of 10^{-5} cm. in height. Using metal and glass surfaces, he found that films of foreign matter as thin as 10^{-7} cm. in contact with the "high points" of the surfaces act as effective adhesives. The tenacity of a monomolecular layer of condensed water vapor is extremely high. To produce maximum adhesion, the liquid adhesive should be applied in sufficient quantity to fill out the irregularities

and provide complete surface contact. Any increase in film thickness beyond that necessary for complete contact weakens the bond. The irregularities should be of a small order of magnitude, for it has been shown experimentally that the strength of the adhesive layer increases as it is reduced to a millionth of an inch or even less.

McBain and Lee (8), discussing the relationship of film thickness to strength, consider that the liquid adhesive is modified and immobilized by contact with the solid surfaces, so that either the range of the molecular attraction approaches the magnitude of the film thickness, or the molecules touching the solid surfaces become oriented, and through chain effect or through micellar linkages, extend this influence through the body of the liquid and subsequently into the solidified film.

Bikerman (13) also believes that the high strength of thin joints is due to long range molecular forces operating either between the surfaces across the adhesive layer or between the surfaces and the interior of the adhesive film. Bikerman also brings up the point that the rate of evaporation will be affected by the thickness of the film and that this rate will in turn affect the structure and stress in the film. In a thin liquid film, the rate is more uniform, and less stress will be produced in the solidified film. In a thick film, a joint may fail within the adhesive film because the cohesive forces of the molecules of the film are not as strong as the forces causing strain.

McBain and Lee studied the strength of joints of varying film thickness formed by using metal surfaces and adhesive gums. The adhesive was allowed to melt completely to form a film, and the metal pieces were pressed together by hand. When aluminum plates and shellac were used, the values given in the accompanying tabulation were obtained.

Thickness of adhesive inches	Joint strength lbs. per sq. in.
Usual (pressed by hand)	2250
0.04	750
0.05	500
0.08	350
0.13	400
0.17	35

Another factor to be taken into consideration as affecting adhesion is the polarity or non-polarity of the adhesive and the surfaces to be adhered. If strips of polar and non-polar solids are immersed in polar and non-polar liquids, it will be observed that there is an attraction between the polar solid and the polar liquid, and between the non-polar solid and the non-polar liquid, but not between the polar substance and non-polar liquid, or the non-polar substance and the polar liquid. Thus if wood, which is normally polar, is immersed in water (polar) and benzol (non-polar), the interfaces will show an attraction between the water and the wood and none between the wood and the benzol.

Similarly, if a plate of bakelite (non-polar) is immersed in the same solvents, the attraction will be between the bakelite and the benzol and not between the bakelite and the water.

De Bruyne (10) has stated as a basic rule of adhesion that with pure or simple substances strong joints can never be made between polar surfaces and non-polar adhesives, or between non-polar surfaces and polar adhesives.

Stamm and Seborg (14), using synthetic resins of decreasing polarity (that is, increasing state of polymerization) in measuring the shrinkage of glued wood joints, found that as the polarity of the resin decreased, the antishrinkage efficiency of the adhesive also decreased. Since the shrinkage of wood is considered to be due mainly to free hydroxyl groups of the cellulose, which through hydration cause swelling and dimensional changes, it is thought that the more polar substances orient themselves in the structure so that the polar groups satisfy a greater proportion of the hydroxyl groups or points of sorption, and thus reduce the tendency to swell. There are, of course, other factors that come into play here, such as greater penetration of the smaller, less highly polymerized molecules into the wood, but it has been shown by these and other investigators that the chief factor is the polarity of the surfaces in contact. Of interest in this connection is the change or reduction in polarity that can be effected in cellulose (wood, paper, etc.) through heating. Apparently the neighboring polar hydroxyl groups form non-polar linkages at elevated temperatures. This is particularly well illustrated in the effect of moisture adsorption in paper (15). It is a fact that should be considered in the study of any problem on adhesives.

This brief theoretical discussion concerns principally molecular aggregates. Starch adhesives are composed chiefly of colloidal units; *i.e.*, aggregates much larger than molecules. The active forces are, therefore, considerably more complicated, though basically similar. From a practical point of view, the following main factors should be considered in determining satisfactory starch adhesives.

1. *Colloidal Lability*—The property of an adhesive dispersion of starch to transform from a hydrosol to a hydrogel, and the great sensitivity of the reversible system, $\text{hydrosol} \rightleftharpoons \text{hydrogel}$, to slight changes are fundamental to these adhesives. By a slight loss of water through absorption or evaporation, by the addition of certain chemicals, by a modification in the operating temperatures, etc., a hydrosol, which is fluid, can be changed into the jelly-like hydrogel. This change is accompanied by an increased rate of tack and better bond. In practice, the system must be adjusted to transform the hydrosol to the particular hydrogel within the tolerance of the operation and the requirements of a particular adhesive.

2. *Specific Bond*—Associated with colloidal lability is the requirement that the adhesive wet the surfaces to be adhered.

3. *Mechanical Bond*—This requires proper viscosity and flow, penetration, and cohesiveness of the adhesive.

4. *Type of Film*—The thickness and structure of the film are important factors.

5. *Deformability*—The adhesive must be able to retain its function in the face of unfavorable conditions. It must be capable of adaptation to film changes subsequent to setting, shrinking, shock, humidity changes, etc.

In connection with these requirements, it should be emphasized that the method of applying the adhesive is very often as important as the properties of the adhesive itself. The conditions for applying the adhesives, by rolls, wheels, sprays, or brush, etc., are quite different in each case, to say nothing of the different conditions with each machine and each plant (16). The experienced chemist, engineer, or operator may well be able to improve upon the operation of any type of adhesive through slight changes in the machine or the adhesive. In many cases, experience and ingenuity will coordinate machine and adhesive in such a way as to produce maximum efficiency at lowest cost.

No attempt will be made to give formulae for the different types of adhesives. Other chapters in this volume are concerned with the constitution of starch micelles and the behavior of these micelles when treated with water. For the purpose of the present discussion we shall simplify the micellar structure and consider it to be built up of two basic units; namely, glucose units combined in a molecular chain, and molecular chains united by primary and secondary valences to form the micelle.

Considering it thus, and keeping in mind, on the one hand, the numerous molecular chains that go into the building of a micelle and, on the other hand, the play of the primary and secondary valences in the spatial arrangement of these chains, we can see that in disrupting the micelles multitudinous fragments can be obtained, each differing in size and in the number of exposed active and potentially active groups. Between the extremes of the undisturbed micelle and the simple glucose units which serve as the basic building blocks any combination and fragmentation are possible. With the uninjured micelle as the starting material, any of the micellar fragments except the final sugar units, such as maltose or glucose, may serve as adhesives. In practice, the two extremes can be represented by starch itself at the upper extreme and by the thin dextrins, such as envelope dextrins, at the lower extreme. In between lies the entire range of other adhesives.

2. Types of Starch Adhesives.

A. *Starch As an Adhesive. Gelatinization in Situ*—The best illustration of the action of unmodified starch as an adhesive is its application in the form of a slurry with water to surfaces, heating to gelatinize the starch, and pressing together the starch-coated surfaces. The starch micelles combine with the water, swell, and burst to form a gelatinous mass producing an extremely strong bond. The process is generally known as "gelatinization *in situ*" and can be applied for bonding purposes to surfaces of paper, fabric, or to any surface that is wetted by an aqueous slurry and to which heat can be applied. Modifying agents may be added to the slurry, which may serve both to improve the working characteristics of the adhesive and the resulting bond. Such agents may be defoamers, plasticizing agents, wetting agents, alkalies, etc.

Recently the method of gelatinization *in situ* has been perfected for use in the manufacture of corrugated and laminated board (17). The process is now widely used in this country and abroad and has replaced sodium silicate, which had been used heretofore in many plants. It is generally referred to as the "Stein Hall process." The essential details in connection with its use in the manufacture of corrugated board are described in the following communication from J. V. Bauer, the inventor of the process.

"Corrugated board is usually made by a continuous two step operation. It consists of corrugating a strip of paper by means of steam-heated fluted rolls, applying adhesive to the tips of the corrugations on one side, bringing a paper liner in contact with the adhesive-coated tips, and forming the bond with the assistance of heat and considerable pressure. This operation may be considered as the first step and forms what is known as single faced corrugated board, consisting of a corrugated strip of paper bonded to a smooth surfaced strip. This phase of the fabrication is performed on a machine known as a 'Corrugator' or 'Single Facer.' The second step of the operation consists of applying an adhesive to the tips of the exposed corrugated surface, bringing a liner in contact with it, and forming the bond with the assistance of heat and just sufficient pressure to hold the paper in contact without deforming the corrugations. This second phase of the fabrication is performed on a machine known as the 'Double Facer.' The result of these two operations is a stiff paper board comprising two smooth outer paper surfaces bonded to an inner core of corrugated paper. The resultant corrugated board is cut into pieces of the desired length by means of a cut-off knife located at the discharge end of the 'Double Facer.'

"In the manufacture of corrugated board the adhesive problem is considerably more difficult than that of bonding two or more smooth surfaced strips of paper, as only a very moderate degree of pressure can be used to assist the formation of the bond between the single faced board and the second liner because of the danger of crushing the corrugations. As a result points of poor contact develop where the tips of the corrugations do not make perfect contact with the liner. It is therefore necessary to apply a sufficient amount of adhesive to the tips of the corrugations to fill in completely these areas of poor contact. Because of these adverse conditions, it has always been customary to heat the board during fabrication in order to cause the adhesive to set up as rapidly as possible. When the board passes from the double backer section of the corrugating machine to the cut-off knife, the adhesive bond must be sufficiently set up to hold the liners and corrugation together so that the board assembly can be cut into sheets and stacked.

"Until the advent of the 'Stein Hall process,' sodium silicate was the only adhesive of low cost that met these requirements to a satisfactory degree. Conventional starch or dextrin adhesives were not found to be suitable for this purpose because of their comparatively slow speed of setting. The reason for the superiority of sodium silicate adhesives over the conventional types of starch adhesives for this purpose lay in the fact that sodium silicate adhesives develop

a very decided increase in viscosity with a small loss of their moisture content, whereas the increase in the viscosity of conventional starch or dextrin adhesives is relatively small, with a corresponding loss in moisture content.

"In the case of starch adhesives gelatinized *in situ* the increase in viscosity which results in the initial bonding effect is caused by the gelatinization *in situ* of the raw starch component of the adhesive when the board assembly is heated on the machine. It is apparent therefore that the time required to form the initial bond with the '*in situ*' type of adhesive is not dependent on the loss of moisture from the adhesive, but is dependent on the time required to heat the paper and adhesive assembly to the gelatinizing temperature of the suspended raw starch. Because of the great extent to which the viscosity of the adhesive can be increased by this means, it is possible to use from 3 to 5 parts of water to 1 part of solids in this type of adhesive and still obtain a sufficient increase in viscosity upon the application of heat to bond the paper satisfactorily when it comes off the machine. When applied to the paper, the '*in situ*' type of adhesive is in a free-flowing, relatively non-adhesive form but is subsequently converted, while between the paper plies, to an extremely viscous condition when the paper assembly is heated above 150° F.

"The most commonly used adhesive of this type consists of ungelatinized starch suspended in an aqueous solution of a gelatinized starch product having sufficient viscosity to keep the raw starch in suspension and to cause the adhesive composition to be picked up and transferred properly on the applicator rolls of the machine. The gelatinized medium is referred to as 'carrier.' In addition to the carrier medium and the starch component of the adhesive, sodium hydroxide is usually included in the composition to lower the gelatinization temperature of the starch. In addition to sodium hydroxide, borax is also used to increase the degree of viscosity developed by the starch when it becomes gelatinized.

"Unmodified starch or partially modified starch may be used as the ungelatinized starch component provided it will form a viscous gel when cooked with 4 or more parts of water. It is this portion of the adhesive which when gelatinized *in situ* is responsible for the main bonding effect. This portion of the adhesive should be present in the proportion of at least 15% of the total solids of the composition if an effective result is to be obtained. It is general practice, however, to use a much greater proportion of unconverted starch than this specified minimum amount. Starches generally gelatinize at temperatures of 150° F. or more. Inasmuch as a gelatinization temperature of lower than 150° F. is desirable in order to obtain a more rapid formation of the bond, this normal gelatinization temperature of starch may be artificially lowered to the desired degree by the use of controlled amounts of a starch-gelatinizing agent such as sodium hydroxide. The carrier medium functions essentially to keep the raw starch component in suspension and to enable the composition to be picked up and transferred to the paper by means of the applicator rolls of the machine. It should be sufficiently dense and viscous to keep the adhesive mixture from soaking too rapidly into the paper and yet be fluid enough so that the adhesive may have the proper flow

characteristics to be handled well in the adhesive pans and adhesive circulating systems.

"Sodium hydroxide because of its effectiveness and low cost is the chemical generally used as a means of lowering the gelatinization temperature of the raw starch component of the adhesive. The amount of sodium hydroxide used to obtain this result depends to a great extent on the proportions of the various other components of the adhesive and on the type of raw starch used. For high speed operation, it is desirable to operate with the gelatinization temperature as low as possible but not so low that premature gelatinization of the adhesive occurs in the paste pans of the machine. The general practice in corrugating is to operate with a gelatinization temperature of between 138° and 145° F. Borax is used in the adhesive formula, because it greatly increases the viscosity developed when the raw starch component is gelatinized. It also acts as an effective buffer for the sodium hydroxide.

"The preferred method of handling this type of adhesive is to use a continuous circulating system in which an excess of adhesive is pumped from the storage tank to the adhesive pans and the overflow pumped back to the storage tank. The temperature of the adhesive in the circulating system is thermostatically controlled to between 100° and 110° F. by means of hot water heating coils in the storage tank. This method of handling results in a uniform temperature of the adhesive regardless of weather conditions and avoids fluctuation of viscosity due to temperature changes. It also eliminates premature gelatinization of the adhesive in the adhesive pans. The proximity of the adhesive pans to the heated portions of the corrugating machine makes such premature gelatinization of the adhesive quite likely unless it is continuously circulated.

"The paste-mixing equipment used for preparing the adhesive in a corrugating plant usually consists of a primary mixing tank in which the carrier portion of the adhesive is prepared, a secondary tank in which the raw starch portion of the adhesive is dispersed and subsequently mixed with the carrier portion, and a storage tank for the finished adhesive.

"It is desirable that the paste be mixed for a total of 20 min. or more after the carrier has been added to the secondary tank. This mixing breaks down the false body of the paste, so that by the end of this time it reaches its true viscosity and will have little tendency to further breakdown in the line. The gelatinization temperature of the paste should be occasionally checked and if necessary adjusted by using more or less sodium hydroxide. As a general rule the gelatinization temperature may be as low as 138° F. without producing serious gelatinization in the paste boxes, but if trouble occurs the gelatinization temperature may be raised by reducing the amount of sodium hydroxide. From the standpoint of speed of production, it is desirable to run with as low a gelatinization temperature as possible, with 138° F. being considered as the minimum safe temperature. The viscosity of the paste may be adjusted to suit plant conditions by varying the amount of carrier starch, and the paste should give a viscosity of about 27 sec. on a standardized pipette used for this purpose."

B. Starch As a Binder in Molded Products—Starch is used with mixtures of wood flour, pulp, clay, and other fillers (singly or in combination) as a binder in the manufacture of dolls, buttons, wooden heels, and other molded articles for which the use of synthetic resin is unsuitable or too costly.

In the "cold molding" process, the filler and starch are mixed together in a dough mixer or other appropriate mixing equipment and steam is injected into this mixture. The heat and condensed water gelatinize the starch, forming an intimate mixture of filler and binder; or the starch is first gelatinized with the water and the filler mixed into it to form a brittle "dough." In either case, the mixture is usually passed through a screen to break up lumps that may have formed, and is molded into the desired shape in steel molds under pressure. The molded articles are permitted to dry out slowly, preferably in chambers controlled for temperature and humidity. Controlled drying is necessary, for if the molded articles are dried too fast, the outer surfaces will harden and prevent the escape of water vapor from the interior. This will result in blow holes or, if the pressure is great enough, in cracks. On the other hand, if the drying is too slow, the compressed particles will tend to pull away from one another, resulting in a product which is crumbly.

Tapioca, because of the gummy characteristics of its gel, is most suitable as a binder. However, other starches can be used. The ratio of starch to filler will vary, depending on the type of filler and to some extent on the starch. With wood flour, a satisfactory ratio is 40% of starch and 60% of filler. The amount of water has to be adjusted so that the proper binding is obtained without oozing of moisture under pressure.

In the "hot molding" process, the filler and binder are mixed with water in the proper proportion, and the mixture is compressed in heated molds or extruded through heated tubes. The heat and pressure act simultaneously to gelatinize the starch and to mold it into the desired shapes. The drying is done in the usual way.

C. Starch in Veneer Glues—Starch is widely used as a veneer glue. However, while the starting point is raw starch, the finished glue represents starch which has become highly modified during the process of glue preparation. Unmodified starch requires a large amount of water to form a workable hydrosol. Such a glue contains comparatively little solids and, upon drying out, forms a thin glue film and a weak joint. Also, the large amount of water causes warping and operating difficulties. Therefore, it is necessary to modify the starch so that it is dispersed in a comparatively small amount of water. At the same time, it must be so modified that the finished glue has the proper viscosity. Should it be too heavy, it will not soak into the fibers and pores of the wood to form a good mechanical joint when pressure to the wood surfaces is applied. If too thin, the pressure when applied will cause the glue to penetrate into the wood too deeply and form what is known as a "starved" joint. The viscosity must also be adjusted so that the glue feeds properly from the rolls and the glue film transfers properly from one surface to another if only one surface is glued.

Assembly, the type of process used (cold or hot), and conditions at the plant determine further necessary variations.

Tapioca is considered the best base for veneer glue. Ordinarily 2 to $2\frac{1}{2}$ parts of water are used for every part of tapioca (by weight). If the agitation in the cooking kettle is good, the glue can be reduced further in viscosity through agitation, in which case the water can be reduced to as little as $1\frac{3}{4}$ parts. The tapioca and water are made into a slurry in the cooking kettle, and sodium hydroxide, dissolved in a small amount of water, is added slowly and gradually to the slurry while the latter is being agitated. The temperature is then raised and the mass heated at about 170° F. until the tapioca is thoroughly gelatinized. The amount of sodium hydroxide used is usually from 3 to 4% of the weight of the tapioca. Its effect is to produce a glue which is smoother and gummier than one made without it. It also undoubtedly acts on the wood cells to expand them and perhaps partially digest them so as to form a more intimate union between the wood surface and the glue. Sodium hydroxide, however, has the disadvantage that it tends to discolor certain woods. Care should therefore be taken in the proportion of the alkali used, particularly where the gumming has to be done on thin veneer. Chemicals other than sodium hydroxide, and oxidizing agents in particular, are also frequently used. These serve to modify the viscosity and properties of the glue still further.

Potato starch can be used in very much the same way as tapioca except that more vigorous agitation or longer agitation may be necessary in order to reduce the glue to the desired viscosity. With corn starch, larger amounts of water must be used. Generally, larger amounts of alkali are also used. This, of course, means longer drying time and greater chance of warping and staining.

Glues made from corn starch also have the tendency to set back on standing; that is, the hydrosol will upon standing be transformed into a hydrogel. Agitation will reverse the hydrogel into the hydrosol. Frequently a small percentage of sulfonated oil added to the adhesive will prevent this "pasting back."

D. Envelope Dextrins—Envelope dextrins represent the lower extreme of starch decomposition referred to previously. Here, the micellar aggregates of the starch have been highly disorganized and the molecular chains greatly shortened through scission and hydrolysis. The resultant products, which are "canary" dextrins, are highly soluble in water at room temperature, 95% or over, and exhibit little viscosity as compared with the original starch in equivalent concentrations.

The manufacture of envelope dextrins is discussed elsewhere. Suffice it to say at this point that tapioca makes the best base, and that the chief properties to be sought for this type of dextrin through the process of dextrinization are high solubility, low viscosity, minimum reversion, and low percentage of reducing sugars.

Envelope dextrins are also known as seal gums, since they are used for the gumming of the flap or seal of envelopes. The requirements for a satisfactory seal gum are many and exacting. Chief among them are the following: In

operation the gum must dry fast and must not "cotton" nor "sugar." It should form a finished seal which does not "block," nor curl, and which has the desired color, gloss, and smoothness. In use, the seal should absorb water quickly upon remoistening, to form a seal with the body of the envelope which does not separate from it upon drying. Fast drying is of extreme importance to the envelope manufacturer, for it regulates the speed at which the machine can be operated. The envelope machines most widely used are either the Plunger or the Rotary type, and these are generally operated at speeds of about 100 or more envelopes per minute. The drying time is therefore very short and within this short interval the seal must become dry enough not to retack when slight pressure is applied due to the accumulation of other envelopes or to packing. The fast drying is obtained by preparing a highly concentrated dextrin dispersion and by watching the amount of sugar in the dextrans or plasticizing agent added.

"Cotting" and "sugaring" are two extremely troublesome factors often experienced with the Plunger type of machine. "Cotting" is the term applied to fibers of gum formed by the "pickers" traveling from the gumming roll to the paper. The pickers, which pick the gum from the gumming roll and stamp it upon the paper, travel at a great speed, and unless the gum breaks clean from the pickers, fibers will be formed. These fibers accumulate and necessitate stopping the machine from time to time to clean them off; otherwise they may drop on the paper or envelopes. As might be expected, cotting is particularly pronounced on dry days, since the lower humidity causes faster drying out of these strands. It may be prevented or minimized by the incorporation of substances that will make the gum "discontinuous." Thus, the incorporation of a small amount of starch will stop cotting. Unfortunately, it will also dull the seal.

"Sugaring" is the opposite of cotting. It is due to the gum being "short," and instead of fibers being pulled out, particles are thrown off the gumming roll on the envelopes.

The finished seal should have good gloss. This is usually the case when the dextrin is properly made, though only tapioca gives a really high gloss seal. Some envelope manufacturers prefer darker seals than others. The color can generally be adjusted during the dextrinization and also by the use of coloring agents. The seal should be smooth, or the remoistening may be uneven. A mottled seal is not necessarily the fault of the gum. It may be due to the paper, if the latter has a finish that is not easily wetted by an aqueous adhesive. However, if the gum lacks good flow, mottling will result.

The period between the manufacture and the use of an envelope may be as long as 2 yrs., and it is important that the envelope neither seal itself, "block," owing to the high humidity at one period or another during storage, nor that the seal curl. An envelope seal may curl owing to extreme brittleness of the gum. The type of paper used may accentuate "curl" still further. If the gum is brittle and the paper is non-absorptive, the film is entirely on the surface of the paper. This creates a strain between the two surfaces of the paper and causes

excessive curling. If the gum is absorbed into the paper, this strain is somewhat relieved and curling is therefore less. Generally, curling is eliminated and flat seals are produced by the addition of plasticizers, such as glycerol, ethylene glycol, invert sugar, etc. The amount of plasticizer added to the gum must be carefully regulated, or these hygroscopic plasticizers will cause blocking. If glycerol or diethylene glycol is used, about 2% based on the weight of the dry gum is the amount generally required.

Seal gums are composed of dextrans which are highly dispersible in cold water. Nevertheless, the characteristics of the gum and of the seal, such as adhesion and appearance, are greatly improved by preparing the gum with heat so that no undispersed dextrin is present in the finished gum. The general practice is to mix the gum with water in the concentration of about 65% solids for the Plunger type of machine, and in somewhat lower concentration for the Rotary type machine, and heat, with agitation for about 45 min. at 180° F. The dispersion is then drawn off and ready for use. It is important that the prepared gum maintain its viscosity over a reasonable period of time, for the manufacturer does not always use it up within a few days of its preparation. Should the consistency of the gum change during storage, the working characteristics of the product are impaired. For instance, should the gum thicken, the operator will add water in order to reduce the viscosity to the proper working consistency. This, however, will change the percentage of solids, and form a thin seal with poor adhesion.

Canary dextrans of the seal gum type have been discussed in detail because, as mentioned before, they represent starch degraded to the maximum degree for adhesive purposes. These dextrans can be and are used for a number of other adhesive purposes besides envelopes, particularly when quick tack and fast drying are required.

They have, however, certain drawbacks which make them unsuitable for a great variety of other adhesive uses. Chief among their disadvantages is the ease with which these dextrans absorb moisture. Owing to the extreme dextrinization treatment, the dextrin unit is small and contains many exposed hydrating groups which will associate with water vapor in the air. This swells the film and weakens the bond between the two surfaces, particularly when the bond must withstand strain, as in the case of paper bags. Another disadvantage is the extreme brittleness of the gum film. Still another is the fact that these dextrans will function properly only when made up in highly concentrated solutions, which means that their spread, in terms of solids per unit area of surface, is low and the gumming costly. As compared with these envelope dextrans, which take only 1 part of water to 2 parts of solids, adhesives can be prepared from less degenerated canary dextrans, and from white dextrans, or British gums, that will take anywhere from 1 to 5 or more parts of water per part of solids. These variations can be brought about by gentler treatment of the starch micelle, so as to avoid excessive degradation, and by the addition of chemical substances that affect the colloidal characteristics of the micellar

fragments and their peptization by water. They constitute large classes of adhesives and have the advantage of lower cost, greater "deformability," and varying degrees of resistance to reabsorption of water vapor.

E. Envelope Seam Gums—The adhesive used for the sealing of the back seam of the envelope must meet a different set of specifications from that of the seal gum. The drying need not be so fast. As a matter of fact, it is preferable that the gum dry slowly so that no strains are developed in the paper, which might cause loss of shape of the envelope. It must be highly plasticized so that the envelope seam will be flat. It must not soften the paper unduly and cause it to pucker, nor must it cause discoloration of the seam on aging. Above all, the seam must be well stuck.

To meet these specifications, the gum is made from white rather than canary dextrin as the base and is combined with plasticizers. The plasticizer assures a flat seam and slow drying, while the white dextrin gives a strong bond, light color, and also contributes to the slow drying and improved flexibility of the adhesive. The plasticizers used are generally sugar or urea and may represent from 30 to 50% of the weight of the dry adhesive.

The sugar-plasticized gum is the type that has been most generally used until recently. It contains up to 50% of sucrose and is prepared by heating the dextrin-sugar mixture in an acetic acid solution of about 35% concentration. The heating is done at 180° F. for about 30 min. The acetic acid inverts the sucrose, forming invert sugar, which plasticizes the adhesive through its hygroscopic properties. This plasticizing effect is further enhanced by the hygroscopicity of acetic acid itself. For Plunger machines the gum is prepared with about 70% of solids, 20% of acetic acid (56% solution), and 10% of water. For Rotaries, more water can be used. As an adhesive, this type of seam gum is particularly effective, for the acetic acid also attacks the paper fiber somewhat and forms a better bond, particularly when the papers are hard sized. Its chief disadvantages are the odor of the acetic acid, to which the workmen in the envelope manufacturing plants object, and the tendency of the invert sugar to brown on aging and form a discolored seam.

The urea type of envelope seam gum (18) is made with urea as the plasticizing agent. It forms an excellent adhesive and in addition is free from the disadvantages of the sugar gum both as to odor and discoloration. In preparing the gum the heating is done at temperatures lower than those for the sugar gum. Temperatures between 140° and 150° F. are preferred. Higher temperatures start decomposition of the urea and the evolution of copious volumes of gas. The prepared gum contains about 77% solids when intended for use with the Plunger type machine, and for the Rotaries the water is generally increased to about 30%.

The back seam gums, and particularly the acetic acid type, are often used as the adhesive for sealing in the windows of envelopes.

Seam gums, like seal gums, must remain fluid when once prepared and must maintain a fairly uniform viscosity over a reasonable period of time. In some

cases, envelope manufacturers buy their gums in the liquid form. Such solutions, of course, must maintain their fluidity and uniform viscosity over a period of months.

F. Bag Gums—In the manufacture of bags, several types of adhesives must be used. If the bag is single ply, such as grocery bags, sacks, etc., a seam gum is needed to form the seal along the side of the bag, and a bottom gum to seal the folds which make up the bottom of the bag. For a bag of several plies, a ply paste is also necessary to seal the plies together at the seam and at the bottom. Often the seam gum can be used for pasting the plies and for the seams. For forming the bottom, however, a more viscous gum is generally used. For the seams and plies, the adhesive must act considerably faster than for the bottoms, and consequently the seam gums generally contain less water than the bottom gums.

Although bag gums could be prepared with starch as the base and modified with chemicals during the heating, most of these gums are made from white dextrans or British gums. The type of dextrin or British gum used will determine the amount of water needed to prepare a gum of proper working viscosity, tack, drying time, bond, and deformability, and these factors are further modified by the addition of chemicals, such as borax, sodium carbonate, sodium hydroxide, etc.

The chief characteristics necessary for a good bag gum are the proper consistency, so that in operation at high speed it neither "throws" or spatters, nor "strings;" it must not discolor the paper; must possess the right tack for the particular speed of operation; and must, of course, form a strong bond, *i.e.* one that will pull fiber if an attempt is made to tear apart the joined surfaces after they have dried. The selection of an adhesive to meet these characteristics will depend principally on the paper, the type of machine used, and the speed of operation. Thus, for a Potdevin machine, which makes the complete bag in one operation, a seam gum is necessary which will set fast, so as to prevent the seam from moving or slipping under the strain when the bottom is formed a few seconds after the seam is glued. With Smith and Winchester and with Coty machines, on which the seams are made and the bags are stacked for subsequent sealing of the bottoms in a different operation, the seam gum need not set so fast, as the seams have more time in which to form the necessary strong bond.

The paper used is an important factor in determining whether a given adhesive will function satisfactorily. A soft paper is simpler to stick than a hard sized paper, but in any case, the size should be of the type which permits the paper to be wetted by aqueous solutions. If such is not the case, chemicals must be incorporated into the adhesive which will dissolve the size or change the surface so that wetting will take place. As an illustration, carbon tetrachloride will help in the case of wax-coated paper, and sodium hydroxide in the case of rosin-sized sheets. The rôle of chemical substances in adhesives will be discussed in greater detail later in this chapter. However, there are some sizings which remain unaffected by the chemicals that can be used in aqueous adhesives.

A great deal of trouble has been experienced recently with the high wet-strength type of paper when resins have been used for increasing the wet-strength.

Since there are many types of dextrans and British gums, and these in turn can be greatly modified in their characteristics by the addition of chemicals, it is obvious that a large number of bag adhesives is possible. These may vary in water-absorbing characteristics from 1 part of gum to 1 part of water, to 1 part of gum to 5 parts of water, and may or may not require heat for preparation. When heat is required, the heating is generally done at 180° F. for about 15 min., with agitation. Most bag gums are used cold. Sometimes, when quick setting is essential, it is advantageous to use the gum at temperatures from 120–140° F.

Recently a wide demand has arisen for gums that will form water-resistant seams and bottoms. These will be discussed later under water-resistant adhesives.

G. Tube-Winding Gums—Starch adhesives are used widely in the manufacture of paper tubes, paper cans, paper cores, etc. In these operations the paper travels from a roll, is gummed by an applicator roll from the gum box, and the gummed paper is wound on a shaped mandrel into a tube, can, core, etc. The wound product leaves the mandrel and is cut to size after it has traveled a certain distance from the mandrel. In the case of tubes and cans, the bottoms are sealed in a subsequent operation.

The number of plies of the formed article will depend on the number of turns of the mandrel. Sometimes different papers are used for the different plies in order to produce a tube or can that meets special requirements such as grease-proofing, water resistance, etc. The adhesive used for these special papers is usually different, and in such cases each special paper has its own gum box.

There is "spiral" winding and "straight" winding, and there are several different types of machines for each type of winding. These, as well as the paper used and speeds of operation, have an important bearing on the choice of a satisfactory adhesive for a particular tube-winding operation. In all cases, one of the most important factors to be considered in selecting the right adhesive is the distance the tube travels before it is cut off, for it is essential that at the cut-off the plies of the paper have stuck together well so that they will not separate at the edges, forming "dog ears." If the distance is short, say 1 ft. or so, the adhesive used must set very quickly; therefore the adhesive must be one prepared with a base that is highly dispersible in water, such as one of the canary type of dextrans, and the amount of water should be kept down to the minimum necessary for the proper viscosity for efficient operation. About 1 part of gum to 1 part of water is the usual ratio. Often the properties of the adhesive are further modified by the addition of alkalies, which serve to increase the speed of the tack, to get a better "bite" into the paper if the paper has a hard finish, and to permit the use of somewhat larger amounts of water, thus increasing the spread and reducing the cost.

As the distance between the mandrel and knife increases, the time given for the adhesive to set is longer. This means that the adhesive may contain more water and still form a satisfactory bond between the plies by the time it reaches

the cut-off. In such cases, starch decomposition products of lower solubility can be used, as they permit the use of more water in the preparation of the liquid adhesive and result in greater economy in the cost of the glue per unit area of paper.

The amount of water a particular operation will tolerate does, of course, vary and the adhesive may be selected so as to take 5 parts of water or even more per part of solids when the cut-off is very long, 60 ft. or more, and less water as the distance to the cut-off grows shorter. In the manufacture of ammunition tubes for the United States government, the lower and upper extremes permitted (19) are a white dextrin having a solubility of 12 to 20%, and a canary dextrin having a solubility of 90 to 100%. The reason for leaving out the solubility range of 20 to 90% is obscure. There is no justification for it from a technical point of view.

Tube-winding gums, if they are not purchased by the consumer in the liquid state, may be of the "cold water-soluble" type or may require heat for preparation. With the former, all that is necessary is thorough agitation with water; with the latter type, the heating is best done at 180° F. for about 15 to 30 min. These may be used cold or warm as in the case of bag gums. Maintenance of uniform viscosity of the prepared gum over a reasonable period of time is also important.

H. Gummed Paper—The adhesives used for "flat gumming" or for gumming paper which is used for labels, stamps, stickers, etc. are generally of the same variety as those used for envelope seal gums. The requirements for such a gum are very much the same as those enumerated for a good seal gum. The brittleness of the gummed film, which has to be counteracted in an envelope gum, is often an advantage for gummed tape, since in many cases the gummed sheets are run over a "breaker bar" which breaks the gummed film into numerous small fractures. Thereby the uneven strain between the gummed and ungummed surface of the paper is reduced, and the sheet lies flat and has little tendency to curl.

The working viscosity of the gum is generally somewhat lower than that for envelope seals, and the concentration is usually about 50% of solids. The gum, prepared by heating with water at 180° F., is generally applied by applicator rolls from the gum box to the paper and is usually dried by passage through heated tunnels.

I. Paper Tape—Until recently, the gum used on the face of paper tape has been universally of the gelatin variety. The requirements for gummed tape are very severe. It must absorb water evenly and develop even tack, or else the tape will stick only in spots. It must, when moistened, have a certain amount of "slip" to enable the user to adjust it slightly if it has not been placed in the correct position. Its initial tack must be fast, but the gum must set slowly so that its tack is not lost if the operator is slow in using the moistened tape. In other words, it must have good "durability." It must not curl, and last but not least, it must form a good bond with the surface to which it is applied.

Within the past several years, a vegetable gum has been perfected which meets these requirements (20). The success of this development is based principally on the combination of urea with starch decomposition products that are not too highly degraded and, therefore, form stronger bonds and less brittle films than would be the case with canary dextrins. Ordinarily such decomposition products would require more water than could be tolerated in the manufacture of gummed tape. However, through the use of urea, the amount of water is decreased, and dispersions can be prepared which are concentrated enough (about 50%) to impart the necessary working characteristics for the manufacture of gummed tape and still permit the desired speed of drying so as not to reduce output.

Through the use of these less disorganized starch products in combination with urea and other modifying agents a tape is produced which meets the exacting requirements mentioned before. Its freedom from odor is an additional advantage. In cases in which unusual quick tack is required, it has been suggested that it be remoistened with a solution of borax rather than with water (21).

The adhesives used for gummed tape are prepared by heating with water at between 180° and 190° F. for 15 to 30 min. The application is very much the same as that for flat gumming, and the drying too is generally done in heated tunnels.

In the "combined" tape, that is in paper tape which is combined with a cloth backing, the combining adhesive used is generally a dextrin product. The specific requirement is a good bond, but the adhesive must be one that is highly plasticized so as not to make the tape too stiff and brittle; *i.e.*, it must have the proper Elmendorf test value. Urea adhesives have been found quite satisfactory.

The combination of cloth and paper for combined tape may also be accomplished by the use of starch through gelatinization *in situ* (22).

J. Laminating Adhesives—Starch adhesives used for laminating paper or board will vary widely in their composition, depending on the paper or board to be laminated, on the type of laminating machine, the speed of operation, the temperatures, etc. In some cases, dispersions of starch itself may be satisfactory. The laminating adhesive in such a case contains a considerable amount of water, as no working gel can be made from starch in concentrations which are much greater than about 12% of solids. Such a dispersion can be used only when the operating speed is low and the time for drying is long. On the other hand, there are some types of lamination, such as mounting printed paper on a paper board backing, for which the adhesive used should contain comparatively little water, 60% or less. In these types of laminating adhesives, it is not only the speed of tack that determines the type of adhesive, but also the necessity of minimizing warping due to strain between the two types of paper. Generally, with less water there is less warping. In many cases, plasticizing materials are incorporated with the adhesive, particularly when quick tack is not essential.

Between the two extremes, starch on the one hand and a highly degraded dextrin which can be prepared with comparatively little water on the other

hand, lies an entire range of adhesives used for laminating purposes. These vary in the percentage of water needed, in the viscosity, tack, etc., and are dependent on the conditions under which the particular adhesive has to function. As an illustration, for a Parry liner, a machine which is very widely used for laminating purposes, a product which takes 3 parts of water is generally found quite acceptable.

Laminating adhesives may be "cold water-soluble" or may be of the type that requires heat for dispersion. The methods for the preparation of the liquid adhesive from the dry product are the same as discussed previously. Lamination may also be effected by "gelatinization *in situ*" (22) of starch in a manner similar to that described in the earlier discussion.

K. Case-Sealing, Carton-Sealing, and Labeling Gums—The adhesives used for sealing cases, cartons, etc., may contain as a base a white dextrin, canary dextrin, or British gum, the particular base used being dependent on the function of the adhesive. In the compounding of the adhesive, the bases are further modified by the addition of chemicals. When the sealing is done on an automatic machine with a belt conveyer, the adhesive must set very fast if the freshly sealed case or carton travels only a short distance under the pressure of the belt. In such cases, a canary dextrin of high solubility, one that will take comparatively little water, is the one to be used. A suitable adhesive is one that takes about 1 to $1\frac{1}{2}$ parts of water for each part of gum; when the sealing is done by hand or when the carton travels under the belt pressure for a considerable distance, the amount of water may run as high as 4 parts or more for each part, by weight, of the gum. Sometimes, when porous board has to be sealed, it may be advisable to use a fairly dilute case-sealing gum and to thicken it with an inert filler, such as clay, that prevents the adhesive from soaking into the board too freely.

L. Cold Water-Soluble Gums, Box Gums—The term "cold water-soluble" gum is applied to dry adhesives which do not require heat for dispersion. These adhesives are prepared simply by mixing with water and agitating the mixture. In use it is found advisable to let such dispersions age for 24 hrs. or so in order to get maximum efficiency in operation. This, of course, is due to a more complete dispersion and to a stabilized colloidal complex.

As would be expected, the bases for cold water-soluble adhesives are canary dextrans and British gums which are highly dispersible in water at ordinary temperatures. The manufacture of these bases requires considerable skill and the proper type of raw material. High quality tapioca flour is the best. At best, however, complete dispersibility of the dextrin or British gum is difficult to obtain. In practice, these bases are mixed with alkaline chemicals, which assist in the dispersibility and also help modify the adhesive characteristics and the water-absorbing capacity of the finished dispersion.

Cold water-soluble adhesives can be used for most adhesive purposes, bag gums, tube winding, lamination, etc. Their widest use, however, is in the manufacture of cardboard boxes, for which purpose they are employed as adhesives for applying the outer paper wrap to the cardboard. There are two

standard methods for this operation, "stripping" and "tightwrapping," and the adhesives necessary for each are different in their requirements.

"Stripping" is the term used to designate the gluing of the paper wraps to the board by hand or slow machine operation. The speed of the tack is comparatively slow and therefore adhesives can be used that are low in solids, 20 to 25%. "Tightwrapping," however, is a fast machine process, and the rate of tack must be quick. For this purpose an adhesive must be used which contains comparatively little water and which has rather critical characteristics. The Stokes and Smith machine is almost universally used for the "tightwrap" operation. The paper wraps, cut to the proper size for the particular box being manufactured, are gummed and conveyed on a belt conveyer to the operator at the machine. The operator places the gummed wrap in position over the cardboard form, and the gluing is done by the Stokes and Smith machine at a fast rate. The adhesive must be of the proper viscosity so that no trouble is encountered in the gumming of the wraps or in producing a thin film. It must not be too slow in tack or the paper edges turned over the cardboard form will spring back when the pressure of the machine is released. When this is the case, the operation must be slowed down by placing fewer gummed wraps on the conveyer so that each wrap has a chance to "temper" longer before it reaches the operator. At the same time the adhesive must not set too fast or there will not be sufficient tack left by the time the wrap reaches the end of the belt where the operator transfers the wrap onto the form. It may also cause unadhered spots or "blisters." The amount of water in the gum is of importance not only to increase the speed of tack but also to minimize warping. Generally adhesives for tight-wrap papers contain from 40 to 50% of solids.

Of primary importance in selecting the proper adhesive for a particular operation is the nature of the paper and board used. The porosity, size, coating, etc., all have an important bearing on the choice of the proper adhesive.

There are numerous other uses for adhesives besides those enumerated above. To discuss them all is beyond the scope of this chapter.

M. Water-Resistant Starch Adhesives—Recently a wide demand has arisen for starch adhesives which, upon drying, will become water-resistant. Although there has always been some use for such a product, the need for it has become of increased importance owing to the government requirements for fiber cases or cartons that will be water-resistant and that can be used for shipment of goods overseas to the armed forces.

The best results in the development of such an adhesive have been obtained from the combination of a starch base with urea-formaldehyde resins. The combination is not water-proof or water-repellent, as the bonding film will absorb moisture even after it is thoroughly dried, but if the adhesive is properly compounded, the absorbed moisture will not seriously weaken the bond or cause adhered surfaces to fall apart or delaminate, even under considerable strain.

One of the most important points in forming a water-resistant adhesive with a urea-formaldehyde resin is the selection of the proper adhesive base. The

underlying principle for guiding this selection is that the base be as little disorganized as possible. The fewer the free hydrating groups present in the starch product and the fewer the number of primary valences, the less is the quantity of resin required to develop resistance to water. The free hydroxyl or hydrating groups in the degenerated starch micelles are undoubtedly the points of entry from which the micellar aggregates or fragments go into dispersion when mixed with water. It is fairly certain that the urea-formaldehyde polymer combines with the available free groups to block these sorption points. Therefore, the fewer free groups present, the less resin is required to block them. Thus it follows that the less disorganized or fragmented the starch decomposition product used as the adhesive base, the less is the quantity of resin required to produce a finished adhesive which will, upon drying, become water-resistant. In practice it has been found that in manufacturing water-resistant laminated board with raw starch and the method of gelatinization *in situ* (22), smaller amounts of urea-formaldehyde resin can be used to procure products equivalent in water resistance than are required when starch decomposition products are employed. British gums and oxidized starches should be more satisfactory than dextrins of equivalent viscosities, and this too has been confirmed in practice.

The urea-formaldehyde polymer can be of varying degrees of polymerization as long as it is in the "water-soluble" state at the time of its use, although the degree of polymerization has an important bearing on its subsequent reaction with the starch product and the speed at which water resistance is developed (14). Generally, in order to develop water resistance fairly soon after the adhesive is applied, a catalyst is used. The catalyst in such cases is the hydrogen ion. The greater the concentration of the hydrogen ion, or the lower the pH (within certain limits), the faster will polymerization take place, with the simultaneous development of water resistance. Unfortunately, the faster the resin polymerizes, the shorter is the life of the adhesive. As the resin-adhesive combination polymerizes, it tends to become more viscous and pasty and loses the fluidity necessary for its satisfactory application. The rate of the development of water resistance must therefore be balanced against the rate of increase of viscosity of the adhesive, and a satisfactory compromise selected. In practice, it has been found that a pH of about 4.5 to 5.5 will permit the paste to maintain its proper fluidity for a period varying from 8 to 24 hrs., depending on the temperature of operation, the concentration of the resin, and the type of adhesive base used. The proper pH is generally obtained through the use of an acid salt, such as ammonium chloride, aluminum sulfate, etc. The amount of catalyst used is generally 10% of the weight of the resin, although this will vary depending on the type of resin. When the percentage of the resin used is small, it is desirable to increase the percentage of the catalyst up to 25% in order to get the maximum water resistance from the resin-adhesive combination.

Excellent application of the water-resistant adhesive is being made now in the manufacture of corrugated and solid fiber board used for making corrugated

and fiber cases for shipment abroad. The latest government specifications for the water-resistant box read as follows (23):

"Water Resistance—The corrugated fiber board used in making interior packing covered by this section shall comply with the following minimum water resistance requirements:

Ply separation, not more than $\frac{1}{2}$ "

Mullen test (per cent of that specified for normal conditions), 50

Tests to be made after 24 hrs. of complete immersion in water at a temperature of 60–80° F. on specimens 6" \times 10" cut from unscored portions of the container or interior packing. Corrugated fiber board for which no Mullen tests are specified shall meet the above ply separation requirements, and have a Mullen test after 24 hrs. immersion of at least 100 lbs."

These specifications are met by the combinations of starch with urea-formaldehyde in the method of gelatinization *in situ*, or by the combination of a starch degeneration product with urea-formaldehyde in the usual procedure of preparing the adhesive with water by heating. Formulae typical of both of these applications are as follows:

(a) Gelatinization *in situ*: 234 lbs. of carrier are heated at 180° F. in 84 gals. of water, cooled to 140° F., and 126 gals. of water are added. 468 lbs. of starch are then added, and the mix agitated to form a uniform dispersion. The pH is adjusted, if necessary, to between 7 and 8 with sodium carbonate. When the temperature of the mix is about 90° F., the urea-formaldehyde resin is added. After the resin is added and before this mix is used, the pH is adjusted to about 5.5 with an acid salt.

(b) Dextrinized starch is heated with water at 180° F. in a concentration of about 35% of solids. When thoroughly peptized, the pH is adjusted, if necessary, to between 7 and 8, the resin added, and the pH again adjusted to between 4.5 and 5.5 before use.

3. Manufacture of Adhesives. In the manufacture of adhesives, the important points to consider are the type of base, the method of manufacture, and the effect of added chemical agents on the properties of the adhesive.

A. Type of Base—Theoretically, any starch can be used as raw material for the manufacture of the starch derivative to be used as an adhesive base. The types of bases obtained from the different starches are, however, widely different. The starches of commercial importance are, principally, corn, potato, tapioca, and to a lesser degree, wheat, sago, and sweet potato. The structure of the different starches, the differences in chain lengths, straight and branched chains, α -, β -, and γ -amyloses, etc., as well as the structural reasons for the different properties of the different starches are discussed elsewhere. For our purpose here, we need to point out only that tapioca represents the best material for the manufacture of adhesives.

The chief reasons for this superiority of tapioca flour over starches of other origin are that (a) the dispersions of tapioca adhesives are clear, (b) the dispersions maintain a more uniform viscosity over a period of time as compared with dispersions of other starch bases, probably owing to the lesser tendency of

converted tapioca starch to revert as compared with other starches, and (c) tapioca adhesives are, as a rule, gummier than adhesives made from other starches.

Corn starch, which commercially is next in importance to tapioca flour for the manufacture of adhesives, forms products that are opaque and films which are rather dull in appearance. Aqueous adhesives made with corn starch bases have a tendency to form thixotropic gels; that is, the adhesive will, upon standing, change from a hydrosol to a hydrogel which, upon agitation, reverts to a hydrosol (becomes fluid again). This is repeated any time the adhesive is allowed to stand unagitated for several days. The manufacturer using adhesives also finds that with tapioca products he has less trouble in maintaining production because of the lesser tendency toward changes in viscosity. Also, the adhesiveness of corn hydrosols is not as good as that of tapioca. Their consistency is "short" as compared with the "long," gummy consistency of tapioca. Besides, corn starch adhesives generally form more brittle films than tapioca adhesives, particularly when white dextrins are used as the base. In spite of these differences, corn starch is used as a base for many adhesive products when quick tack and high speed of operation are not of paramount importance.

Recently, corn starches modified in micellar structure during their separation from the grain have been used as the raw material for adhesive bases. Various varieties of corn starch have been used as the starting material, including waxy maize. For the latter, especially, claims have been made that it is as satisfactory for many uses as adhesives made from tapioca. However, the work done with it up to now has not been sufficient for thorough evaluation.

Potato starch forms adhesive bases which are satisfactory for many purposes. The adhesiveness of many potato starch bases is nearly as good as that of tapioca flour bases, and the films are clear and glossy. However, potato starch adhesives "set back" (revert) faster than tapioca adhesives. This constitutes a great disadvantage, for, while they can be used satisfactorily at elevated temperatures, it is difficult to use them at room temperature, particularly when the percentage of solids is high. Another, although minor disadvantage of potato starch adhesives is their odor and taste; potato dextrins have a peculiar odor and taste, sometimes described as that of a cucumber, which is carried over into the adhesives. A further drawback is the high price of potato as compared with other starches.

Adhesive bases obtained from wheat starch are similar in the main to those made from corn starch. As compared to corn starch, they have one objectionable feature; that is, foaming. Wheat starch adhesives foam excessively, and even with the use of foam depressants it is not always possible to eliminate the foaming entirely.

The work done with sweet potato starch indicates that it forms excellent adhesives with properties closer to those made from tapioca than to those from corn, wheat, or potato starches. Owing to the comparatively small amount of sweet potato starch available, its use for adhesives has been limited.

Adhesive bases made from starches are usually in the form of canary dextrins (of high solubility), white dextrins (of low solubility), and British gums (of high or low solubility). To a lesser extent they may consist of modified starches, such as thin boiling, oxidized, or enzyme-modified starches.

B. Methods of Manufacture—The principal method for the manufacture of adhesives consists of blending the proper dextrin or British gum bases, obtained by the usual method of roasting starch, with the necessary chemical substances to obtain a final product that will possess the characteristics essential for the particular purpose for which the adhesive is intended. Generally, the user of these adhesives will himself perform the final step in their manufacture; that is, the preparation of the liquid adhesive from the dry by the addition of water and by heating. If the dry adhesive is of the "cold-soluble" type, no heating is needed. In many cases, this final step is also performed by the adhesive manufacturer, so that the consumer buys liquid adhesives which are ready for use as received, with the possible exception of further dilution with water to adjust them to the proper working viscosity.

It is possible in some cases to use unmodified starch as a starting point and modify it during the heating period in water by the addition of chemicals (29). The preparation of veneer glue is an example. However, the controls necessary for preparing good adhesives in this manner are exacting and are difficult to establish in the majority of plants. The manufacturers of liquid adhesives seldom use this method, as it does not lend itself to the manufacture of products of uniformly high quality. Most of the liquid adhesives made by adhesive manufacturers when starch, and not dextrin or British gum, is the starting point, are produced by means of enzymes or acids. With enzymes, the α - or liquefying amylases are used as free as possible from the β - or saccharifying amylases so as to form as little sugar as possible, since the presence of an appreciable amount of sugar is undesirable for most types of adhesives. When enzymes are used with starch to produce adhesives, the starch slurry is mixed with the required amount of enzyme and, after the pH of the slurry has been adjusted to the optimum point for conversion, it is heated at the proper temperature until the desired viscosity is obtained. In determining the best temperature for a particular conversion, consideration should be given to the kind of starch used. The minimum temperature for the conversion must be slightly above the gelatinizing temperature of the particular starch, and differs for the different starches. However, the enzymes must also be selected with care, for the commercial enzymes on the market vary and have their own optimal conditions for best performance, particularly as to pH, temperature, and concentration. In all cases, the enzyme must be inactivated after the desired viscosity is obtained, so that the finished product does not continue to thin on standing. This inactivation may be done with heat, or by chemical means through the use of enzyme inhibitors.

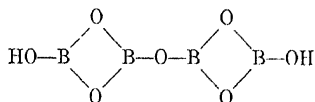
In the preparation of concentrated adhesives from starch, it may be necessary to carry out the conversion in several stages, particularly for corn or wheat starch. In such cases, a conversion may begin at a concentration of 15 to 20%

of starch solids and be allowed to proceed as far as it will go. More starch and enzyme are then added and the conversion continued. This may be repeated until the required amount of solids is obtained. For an adhesive requiring quick adhesion and quick drying the percentage of moisture cannot be much above 60%. In many cases, it is impossible to start out with a 40% starch slurry, and it is therefore necessary to resort to conversion by steps. As is the case with dextrans, or British gums, chemical substances are added to these liquid conversion products to make the finished product. The kind and amount of chemical agents depend on the specifications for the particular adhesive.

There are a number of dry, "cold water-soluble" adhesives, which are known as "drum dried" or "hot roll" products. These are made by heating the proper dextrin, British gum, or modified starch with water, adding the chemicals necessary for the particular adhesive, and then removing the water by evaporation on a revolving, heated drum. Instead of a modified or dextrinized starch, raw starch may be used as the starting point and modification brought about by means of enzymes, or a combination of an enzyme-converted material and a dextrin material can be used. The dry product made from the proper base and containing the requisite chemical additions comes off the roll in the form of flakes and then is ground to the proper particle size for the finished product. One of the important points of superiority of these hot roll products over other "cold water-soluble" products is the ease with which they disperse in water without lumping.

C. Chemical Additions—Borax is the most important single substance among the many used to modify adhesives. The man who first discovered the effect of borax on starch adhesives has made the most outstanding contribution in this field. At the time this discovery was made, the reasons for the effect were obscure. Today we understand these better, though we are still uncertain which of the possible mechanisms plays the most important rôle.

Compounds containing free hydroxyl groups will in aqueous solution combine with some of the water, through hydrogen bonding, to form complex molecules. The water that has entered into chemical combination with the dissolved or dispersed substance is "bound," as distinguished from that portion of the water which is not chemically combined and which thus remains "free" and acts as the solvent. The removal of water from the solvent changes the characteristics of the solution, and particularly the viscosity. When starches or dextrans are dispersed in water, some of the water is chemically bound with the starch or dextrin through the exposed hydroxyl groups, while a portion of it remains as free water. Borax, which is considered the sodium salt of dihydrotetraboric acid, will on solution with water form a certain amount of free acid. The structural formula (24) of the acid may be regarded as



When borax is added to a starch or dextrin dispersion, the resulting effects may be due to the association of the borax or dihydrotetraboric acid molecule with some of the remaining free water, to the coupling of the borax molecules (hydrated or non-hydrated) with the dextrin or starch molecules¹ (hydrated or non-hydrated) through hydrogen bonding, or to both. This results in new combinations having properties that differ in one or more respects from the original mixture. As the number of exposed hydroxyl groups in starch and dextrin may vary greatly, it is obvious that the possible combinations are many.

To the chemist dealing with adhesives, the most important effect of the addition of borax to dextrin or British gum is the resulting increase in viscosity and the "gummier" characteristics. It is possible to take a British gum of high solubility which, by itself, will form a workable hydrosol only in high concentration (*e.g.*, 50% of solids) and, through the addition of borax, form an adhesive which contains only 20% of solids and is equivalent in viscosity and tack to the more concentrated hydrosol. Adhesives treated with borax tack faster, as a rule, than adhesives not containing borax. The bond between the two surfaces to be held together is quicker, thus permitting greater speed of operation by the manufacturer. The reason for the faster tack is probably the comparatively small amount of free or solvent water present in these borated adhesives. As the amount of solvent is small, the dispersed material is nearer the saturation point and is thus very sensitive to further slight changes in concentration. In the use of the adhesive, the loss of a slight amount of solvent water, through absorption or evaporation, is sufficient to transform the hydrosol into a hydrogel. The effect of borax on the adhesive bases varies widely with the type of base used. Little borax is necessary to increase greatly the viscosity of a starch dispersion, although with dispersions of modified starches, dextrans, or British gums, greater amounts of borax are needed.

In the formation of adhesives with dextrans or British gum, the viscosity and tack are increased with increased concentration of borax, up to a certain point. If the amount of borax used is excessive, the resulting dispersion cannot be spread into a smooth continuous film. Apparently too much free water is removed, not leaving enough solvent water to give the liquid the necessary fluidity. The adhesive also loses its tack and becomes rubbery. A partial explanation for this loss of tack is, probably, the lack of free hydroxyl groups necessary for wetting the surfaces and for specific adhesion.

Most adhesives, with the exception of those that are prepared at an extremely high concentration of solids, such as envelope adhesives, contain borax. Borax is essential for the preparation of "cold water-soluble" starch products and for this purpose it is often used in combination with other alkalies. The use of other alkalies with borax modifies to a certain extent the effect of the borax. These alkalies modify the characteristics of the finished adhesive further. Chief among such alkalies are sodium hydroxide and sodium carbonate. Sodium

¹ Borax materially retards the association and spontaneous precipitation of the linear polymer fraction of starch. Ed.

in mind that the starch adhesive is aqueous and therefore the surface-modifying agent should have groups that can associate with water. Another point to be remembered is that too liberal an amount of these wetting agents affects the tack. The material most commonly used is sulfonated castor oil.

E. Plasticizers—A dry vegetable glue film is quite brittle. Even though starch micelles are considered to be made up of molecular chains arranged more or less in loop form, a condition which should tend to impart elasticity, the dry starch films are nevertheless far from flexible. As might be expected, dextrans and British gums which consist of fragmented starch micelles form films which are more brittle than those of the original starch. The starches and starch products made from the different starches differ somewhat in elasticity. Thus, potato and tapioca are considerably more elastic than corn or wheat, but at best the elasticity of the most elastic of the starches is comparatively poor. The brittleness of the vegetable glue film may be reduced somewhat by making the film thin. It is determined also to some extent by the surface on which the film is formed, but any further improvement necessitates the use of plasticizing agents.

The important requisites of plasticizing agents for starch adhesives are that the plasticizer be miscible with water, that it be compatible with the ingredients of the adhesive, and that it should not affect adversely the performance of the adhesive, at least not any more than can be tolerated safely by the particular adhesive to be plasticized. Most of the plasticizing agents used depend on their hygroscopic properties and a few on their fatty characteristics.

Chief among the plasticizing agents which depend on their hygroscopicity for flexibility are glycerol and the related products, the ethylene glycols. The dried film, containing a small percentage of glycerol or ethylene glycol, will hold tenaciously to the associated water molecules and thus will prevent the film from completely drying out and becoming brittle. Should the film become extremely dry, due to particularly arid atmospheric conditions and high temperatures, it will reabsorb moisture from the atmosphere when the humidity is increased. The moisture in the air and the moisture in the glycerol-containing film constantly tend to become equilibrated, and the conditions affecting this equilibrium are determined by the vapor pressures of the system, vapor-glue film, at any one time.

If the film retains or absorbs an excessive amount of moisture, the bond is weakened, and in extreme cases enough water might be present so that the bonded surfaces can be pulled apart without tearing the fiber. Should this excessive absorption take place on a surface film, such as envelope seals, labels, etc. "blocking" will take place. Excessive amounts of glycerol will also tend to reduce the rate of development of tack and the speed of adhesion. It is therefore extremely important to adjust the amount of glycerol added, so as to obtain flexibility without sacrificing strength of bond, rate of tack, or running the danger of "blocking." This quantity is not always easy to determine, since the amount of moisture in the film is dependent on atmospheric conditions.

Glucose and invert sugar are used as plasticizing agents. As with glycerol, the improved flexibility of the film is due to the moisture which it retains or

absorbs. Invert sugar is considerably more hygroscopic than glucose and is therefore superior to it. Neither of them, however, is as good as glycerol or diethylene glycol, principally because they give up absorbed moisture too readily under dry atmospheric conditions and reabsorb it too slowly. Sorbitol is considered superior in these respects and its use has been advocated. The effect of the sugars on the bond, rate of tack, "blocking," etc. must be considered just as in the case of glycerol. The sugars have the additional disadvantage that they tend to develop color with age. This brownish coloration is undesirable in some applications.

The use of glycerol, ethylene glycols, sugars, etc., is not advisable with borated gums, since these products interact with the borax to form new products, the properties of which may not be consistent with the requirements of the adhesive. Other substances used as plasticizers because of their hygroscopicity are calcium chloride, ammonium nitrate, urea, sodium acetate, etc.

Sulfonated oils or fats, sulfated alcohols, and soluble soaps are examples of substances used for plasticizing in which the effect is not due to the retention or absorption of moisture. These substances tend to impart a permanent flexibility independent of atmospheric conditions. Their chief drawback is that generally the amounts necessary to give appreciable flexibility, say, about 3% of sulfonated oil, decrease the speed of tack and weaken the bond.

F. Defoamers—The foaming of adhesives is one of the most troublesome factors in adhesive operations. If foam collects in the gum box, the gum will not transfer properly onto the rolls; it will be full of air bubbles and poor in solids, the operating speed will be reduced, the bond will be poor, etc. It is therefore extremely important that adhesives subject to foaming contain defoaming agents to counteract the accumulation of foam.

Foams are dispersed systems of globular liquid films containing gases. These systems are formed in adhesives sometimes during the cooking, in which case the gas may be water vapor. More often, however, they are formed in use, owing to the incorporation of air into the surface of the liquid through the revolution of the rolls. If the systems are unstable, then the films break, and no accumulation of foam takes place. If they are stable, the foam accumulates and becomes troublesome. Development of foam and its relative stability are due to surface and interfacial forces and can therefore be greatly affected by the presence or introduction of surface-active materials.

The formation of foam is generally due to surface-active substances, foaming agents, which are present in the adhesive as impurities. Thus, the excessive foaming of adhesives made with a wheat starch base as compared with the foaming of adhesives made from tapioca is most likely due to traces of gliadin present in the wheat starch. The tendency to foam may be further increased by the viscosity of the dispersion, which causes air bubbles to be trapped and slows their rise to the surface where bursting and escape of the gas or air takes place. To prevent the formation of foam, other surface-active materials must be added which will modify the surface tension and interfaces in such a way as

to counteract the action of the surface-active materials responsible for the foaming. Chief among the defoamers are alcohols, ethers, esters, fatty acids, oils or fats, and sulfonated fatty acids, oils, or fats. The higher alcohols, such as capryl, heptyl, and nonyl are effective foam depressants. Their action is due to the fact that they form delicate films of low viscosity which tend to replace the more persistent films. They are effective in most types of solutions, particularly in those containing proteins, and exceedingly small amounts are sufficient to destroy accumulated foam and prevent its re-formation.

Oleic, stearic, and palmitic acids are effective foam depressants in many cases. Vegetable oils and sulfonated vegetable oils are also useful. The effects of these substances may be due either to differences in surface tension between the surface-active materials (*e.g.*, the added substance may lower the surface tension more than the substance already present at the interface and will displace it from the interface) or to the presence of polar and non-polar groups which take a definite surface orientation, or to both. The amounts of these materials necessary to prevent foaming may vary from 0.1 to 0.5%, based on the weight of the solids. The amount is rather important, as in some cases an excessive amount of defoamer will cause re-formation of foam. The phase may change from one consisting of water saturated with the defoaming agent to that of the defoaming agent saturated with water.

Tributyl phosphate and tributyl citrate have also been used successfully to prevent foaming, in the amounts of 0.1 to 0.25%.

G. Preservatives—Preservatives are generally unnecessary in aqueous adhesives when the percentage of solids is high. Envelope adhesives, containing about 65% of solids, seldom, if ever, require a preservative. Apparently there is not sufficient free water available to encourage the propagation of the stray molds or bacteria that may enter it from the air or from the vessels or materials used.

Adhesives that contain appreciable quantities of chemicals, such as borax, even though they may be made up with a fairly large amount of water (70 or 80%) are also seldom subject to the action of molds and bacteria. Nevertheless, there are occasions when even envelope gums or borated gums become favorable substrates for the activity of bacteria and molds. This is evidenced by a lowering of the viscosity and the development of a sour odor. The infection is due to the presence of a particularly virulent strain of bacteria or mold with which the adhesive dispersion has become contaminated, or one which has been cultured in the residues of the adhesive in the containers and has developed high tolerance to that particular composition. The usual remedy in such cases is to clean thoroughly all the vessels used in preparing and storing the adhesive with a hypochlorite or other such bactericidal compound.

When the concentration of solids is low (*e.g.*, less than 30%) and no chemicals are present which create an unfavorable medium for living organisms, preservatives should be incorporated, particularly if the adhesive is not used up within a short time. It is extremely difficult to make definite recommendations on the

type of preservative substances to be used. This is readily understandable when we consider the great variety of strains of molds and bacteria which vary in their response to different chemicals. What may be toxic for one type of organism may be entirely harmless for another. It is suggested, generally speaking, that a preservative be used which will affect both molds and bacteria, and if only one substance will serve each purpose for a particular solution, then two different chemical agents should be used, one specific for molds and the other for bacteria.

Of the various chemical compounds, mercurials are among the best. They are effective against most types of organisms. Chlorinated phenols are quite effective. A small amount, 0.2%, of the sodium salt of *o*-phenylphenol (Dowicide A) or of the sodium salt of pentachlorophenol (Dowicide G) offers good protection. Formaldehyde, in percentages of 0.5 to 1.0, is widely used. It is particularly effective against molds. Copper sulfate, to the extent of about 0.2%, zinc sulfate, fluorides, benzoates, and phenols are frequently used (28).

In selecting the proper preservative, consideration should be given not only to the protection of the adhesive, but also to the possible toxic effect of the particular chemical on the operator in the plant and the possible implications due to its presence in the finished product.

H. Color—The color of adhesives is of some importance when they are used on exposed surfaces, such as on labels, envelope seals, etc. When the adhesive is used for bonding two surfaces, color is of little concern unless the laminated material is white and it is important not to reduce the whiteness through the use of a dark adhesive.

The color of the adhesive can be regulated to a large extent during the manufacture of the base. However, if adjustments in color of the finished adhesive are necessary, darker shades may be produced by the use of caramel coloring, and lighter shades by bleaching. The more effective bleaching agents used are sodium bisulfite if the medium is acid, or perborate, if the medium is alkaline. From 0.1 to 0.25% is generally sufficient. Hydrogen peroxide is also widely used.

I. Emulsions—Mention has been made before that the incorporation of sodium hydroxide into an adhesive solution is of considerable help when the surfaces to be adhered are sized with rosin. In such cases, the alkali acts on the rosin by forming a soluble salt which disperses through the solution, thus permitting the adhesive to reach the paper fibers. When the paper or board is sized with oil, wax, insoluble soaps, or synthetic resins, alkalies may have little effect. In such cases it is necessary to incorporate a solvent which will dissolve the oil, wax, or soap layer and thus expose the paper surface. The solvent to be selected should be one that will solubilize the particular material with which the paper or board is sized, and preferably one that is not miscible with water. A solvent that is miscible with water is apt to be too highly diluted with the water to be effective, unless large amounts of it are used. Best results are obtained through the use of an immiscible solvent by emulsifying it with the aqueous adhesive into an emulsion that does not possess high stability. When the adhesive is applied to the surface, the change in the system due to evaporation,

etc., is sufficient to break the emulsion and permit the solvent to act independently on the surface to dissolve off the protective film. As an illustration, it is possible to form a good bond with an aqueous adhesive on a waxed surface by incorporating a small amount of carbon tetrachloride (about 10%) and emulsifying it with sulfonated castor oil (about 1%).

J. "Fluidifying" Agents—Many adhesive solutions tend to "paste back" and lose fluidity within a short time after they are prepared. This is particularly troublesome when the base of the adhesive is a white dextrin or a thin boiling starch. A pasty adhesive cannot be used in operations for which a low viscosity is necessary, and if this "pasting back" occurs before the adhesive is used up, it is a source of many difficulties for the operator. Frequently, a solution may thicken gradually for several days before completely "pasting back." In such cases the machine operator will attempt to dilute the viscous solution with water in order to bring it to the right viscosity for machine operation. This, however, lowers the content of solids of the diluted adhesive, reduces the speed of tack, and slows up operation.

To overcome this tendency to "paste back," and to maintain greater uniformity in the viscosity of the adhesive solution during its life, various chemical substances can be used. Salicylic acid and related compounds are quite effective, even in small percentages. However, because of their phenolic nature they usually develop color, which is objectionable in many cases. They are also comparatively costly.

Formaldehyde in the proportions of about 2 to 3% is also effective in some cases. Boric acid, up to 5%, is widely used. It has been claimed that inorganic salts, such as potassium chloride, sodium sulfate, etc., serve to keep a solution fluid. Acetamide and compounds related to it have been advocated, while urea, potassium iodide, and thiocyanates also have been found of definite value in minimizing changes in viscosity in adhesive solutions.

The underlying principle is most likely due to the reactive groups reacting in such a manner that little association with water can take place, thus leaving the free water to act as solvent.

4. Conclusion and Acknowledgment. It has been quite a task to condense the subject of starch adhesives into the space of one chapter. It has necessitated discussing briefly subjects that merit more detailed treatment. In particular it is regretted that more space could not be devoted to the relationship between the adhesive and machine and the importance of coordinating the one with the other. It is hoped, however, that this chapter will serve as a stimulus to the chemists working on starch adhesives for a more scientific approach to problems concerning adhesives.

Appreciation is expressed to Mr. J. V. Bauer for his communication on corrugating adhesives, and to Mr. John Mahlmann for gathering some of the references cited.

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